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LABORATORY MANUAL
FOR THE
DETECTION OF POISONS
AND
POWERFUL DRUGS

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AUTHOR'S PREFACE

In the latest edition of this book, the original arrangement of subject-matter has been retained but each chapter has undergone thorough revision. Several new subjects and methods have been incorporated and the number of illustrations has been considerably increased. The chemical literature has received full consideration and also, as far as seemed necessary, that relating to matters of pharmacologico-chemical interest. In rendering expert opinions, the forensic chemist in many instances will find these references to original literature very useful and by studying them students should acquire a far better knowledge of the subject. Information with regard to the physiological action of the more important poisons and drugs should prove of service to many chemists and the same may be said of the matter pertaining to the changes which these substances undergo in the animal body and to the forms of combination in which they are eliminated. Students should not be permitted to perform their laboratory work in a purely perfunctory manner but they should be encouraged to take advantage of every opportunity to acquire at the same time a theoretical knowledge of the subject by studying methods of preparation and the chemistry of the more important substances, the alkaloids in particular.

The new edition of this work, in comparison with what has gone before, is much more extensive in its scope but the subject-matter is so distributed as not to be detrimental in any way to the book as a whole. The student in most instances will not be concerned with more than the first three chapters, although a student of pharmacy may also add the sixth chapter dealing with the methods used in assaying potent drugs and their preparations.

WILHELM AUTENRIETH.

FREIBURG IN BREISGAU.

67872

TRANSLATOR'S PREFACE TO THE SIXTH EDITION

This edition of "*Detection of Poisons*," though differing hardly at all from earlier editions in method of treatment, presents notable changes as far as contents are concerned. Many new substances and methods have found places in its pages. The first chapter which is confined to volatile poisons now includes several related phenols in addition to carbolic acid, and other compounds appearing here for the first time are butyl chloral hydrate, isoamyl alcohol, formaldehyde and formic acid. Added to the non-volatile organic substances, which go to make up the second chapter, are lactophenine, salol, aspirine, and the alkaloid scopolamine. The third chapter treating of metallic poisons has undergone practically no change.

By far the greater part of the new matter is to be found in the last three chapters of the book. In the fourth chapter, which is concerned with such substances as find no place in the first three groups of poisons, the author has introduced such inorganic compounds as hydrofluoric acid and fluorides, bromine and bromides, iodine and iodides, hydrogen sulphide, sulphur dioxide and sulphurous acid, boric acid, nitrous gases and nitrites. Salvarsan and derivatives are appropriate additions to the organic arsenicals. Synthetic hypnotics and sedatives, represented by such compounds as propanal, luminal, medinal, dial, adaline, bromural, neuronal, diogenal and nirvanol, have received attention. Aconitine, pseudoaconitine, arecoline, johimbine and lobeline are selections from the less common alkaloids. Such cocaine substitutes as novocaine, tutocaine, psicaine, orthoform (new), and stovaine have been included together with the morphine derivatives, dionine and heroine. The subject of digitalis glucosides has received fuller treatment and been supplemented by the addition of the related strophanthin. Even the unusual toxic substances isolated by Wieland from the skin of the toad have been made a part of this lengthy chapter.

Certain apparatus and special analytical methods form the subject-matter of the fifth chapter. Different forms of extractors

are described and particular attention is paid to colorimetric estimations by means of the author's colorimeter. The usefulness of microsublimation *in vacuo* as a means of detecting alkaloids is emphasized. Special methods of analysis are divided into two groups. The first group comprises methods applicable to the examination of inorganic substances such as phosphorus, arsenic and certain metals. The second group deals with the examination of organic substances among which are hydrocyanic acid, methyl alcohol, formaldehyde, several important alkaloids, and chloral, carbolic acid, pyramidone and veronal as found in urine. In conclusion this chapter takes up methods of examining inflammables containing phosphorus and official German methods employed in detecting arsenic and tin in textile materials.

As in former editions the sixth chapter is given over to quantitative methods of pharmaceutical interest. Many methods of the German Pharmacopoeia employed in estimating alkaloids are given in full. At the same time many non-official methods of estimating alkaloids and other substances, several of which appear for the first time, are included.

The author concludes with an Appendix in which he gives at considerable length the Meyer-Overton theory of narcosis, the investigation of the constitution of colchicin and colchicein by Windaus, and the results of Willstätter's study of the cleavage-products of scopolamine.

William H. Warren.

Worcester, Massachusetts.

INTRODUCTION

Upon the basis of chemical behavior during analysis, nearly all the commoner poisons and potent drugs may be put into one of the three following groups:

I. Members of this group distil with steam from acid aqueous solution. Herein are included yellow phosphorus, hydrocyanic acid, carbolic acid, cresols, naphthols, chloroform, iodoform, chloral hydrate, aniline, nitrobenzene, methyl alcohol, ethyl alcohol (alcohol), amyl alcohol, formaldehyde and carbon disulphide.

II. Members of this group are organic substances non-volatile with steam from acid aqueous solution. Hot alcohol containing tartaric acid extracts them from extraneous matter. Alkaloids, many glucosides and bitter principles, as well as various synthetic organic compounds used in medicine, such as antipyrine, acetanilide, phenacetine, pyramidone, sulphonal and veronal, are found here.

III. All poisonous metals belong in this group.

According to this classification, toxicologico-chemical analysis is divided into three distinct parts, each with its own special chemical procedure. A few poisons, such as mineral acids, caustic alkalies, potassium chlorate and oxalic acid, can not be placed in any of these three groups because of differences in solubility and other peculiarities. Such poisons can be detected only by applying special tests to the original material and sometimes by employing special methods.

In a toxicologico-chemical examination, material to be tested for all known poisons should be divided into at least four parts. This is necessary when, for example, poisoning is suspected but the autopsy and medical history of the deceased fail to furnish the chemist with clews as to how to proceed in his chemical examination of the given material for poison. One portion should be examined for poisons volatile with steam from acid solution (I) and at the same time for metallic poisons (III). After removal of volatile substances by distillation, the residue can be tested for such metals as may be present. The second portion should be examined for organic substances, such as alkaloids, glucosides and synthetic

drugs (II). The third portion should be examined for those substances that find no place in the three main groups of poisons. Finally, a fourth portion of material should always be reserved for confirmatory tests, in case they are necessary. Such tests must be made when the first examination fails to establish with certainty the nature of the suspected poison, or when it has gone wrong. If only a limited amount of material is available, one portion may be used in testing for all three main groups of poisons. In that case the residue in the distillation-flask should be divided into two unequal portions. The larger should be tested for non-volatile organic substances (II) and the smaller, to which may be added the extraction-residue from II insoluble in alcohol containing tartaric acid, for metallic poisons (III). Also in this case it is advisable to reserve a small portion of the original material for subsequent tests.

In some instances it is impossible to conduct a toxicologico-chemical examination according to the fixed plan just outlined or by any other plan. Frequently the forensic expert cannot formulate a plan until he has taken all attendant circumstances into consideration. Before entering upon an investigation of this character, the chemist should first thoroughly and critically study all papers relating to the case, such as the medical history, if there is one, and the report of the autopsy. Particular attention should be paid to drugs administered during illness.

Poisons differ widely in their behavior in the animal organism. Some are retained by certain organs, such as the liver, whereas others pass into the blood and are rapidly eliminated in the urine. For these reasons it would obviously be futile in many cases to remain satisfied with an examination of one particular organ for all poisons that might come into question.

The systematic general procedure employed in testing for poisons often need not be carried through to completion as, for example, in a toxicologico-chemical examination where the search from the outset can be limited to a definite poison.

The custom is to deliver to the forensic chemist in separate containers all organs and animal fluids, such as parts of the stomach, intestines and contents, pieces of liver, kidney and spleen, and also blood and urine. Each part should be examined by itself, even when all the parts belong to the same case. The only exception to this rule is a case demanding the speediest information possible as to presence of poison in the parts of the given cadaver. In order to

answer such a question as quickly as possible, the author takes a good average sample of each part, organs as well as body-fluids, mixes them and examines the mixture for poison by the systematic general procedure. If this preliminary examination discloses a definite poison, it is sometimes, though not always, of interest to determine in what organs or body-fluids the given poison is located.

Before they are subjected to chemical examination for poison, organs such as liver, spleen, kidneys, heart, brain, or pieces of the stomach and intestines with contents should be brought to as fine a state of division as possible. For this purpose strong anatomical scissors that are sharp and thoroughly clean are very useful. Organs may be held by forceps or tongs made of German silver or nickel. A small meat-machine that has been carefully cleaned is also very convenient for this purpose.

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DETECTION OF POISONS

CHAPTER I

VOLATILE POISONS

Phosphorus and Other Poisons Volatile with Steam from Acid Solution

Many materials acidified with tartaric or dilute sulphuric acid and distilled with steam yield distillates containing those substances that are volatile under these conditions. As far as toxicological investigations are concerned, the most important compounds of this class are:

Phosphorus,	**Chloroform,**	**Ethyl Alcohol,**
Hydrocyanic Acid,	**Chloral Hydrate,**	**Methyl Alcohol,**
Carbolic Acid,	**Butyl Chloral Hydrate,**	**Amyl Alcohol,**
Cresols,	**Iodoform,**	**Formaldehyde,**
Thymol,	**Nitrobenzene,**	**Acetone,**
Naphthols,	**Aniline,**	**Formic Acid.**
	Carbon Disulphide,	

PHOSPHORUS

Scherer's[1] Preliminary Test for Phosphorus.—This test should always be made before testing for yellow phosphorus by distilling with steam. The principle of this test is that yellow phosphorus is volatile on gentle warming, or even at room temperature. Its vapor will blacken silver nitrate either from formation of silver phosphide, or because phosphorous acid produced by oxidation of phosphorus reduces silver from silver nitrate. This test, however,

[1] J. Scherer: Detection and Estimation of Phosphorus and Phosphorous Acid in Poisoning Cases. Ann. d. Chem. 112 (1859), 214.

may lead to error, for the material may give off hydrogen sulphide which will also blacken silver nitrate paper.

To establish the presence or absence of hydrogen sulphide, the vapor given off should be tested with "lead paper." Hydrogen sulphide will blacken this paper but phosphorus will not. Addition of cadmium sulphate to the material will remove hydrogen sulphide but have no effect upon phosphorus.

Procedure.—Place the finely divided material in a small flask and cover with water, if a sufficient quantity is not present. Cut a V-shaped slit in the cork and place the latter loosely in the mouth of the flask so that the two strips of filter paper hang free (Fig. 1).

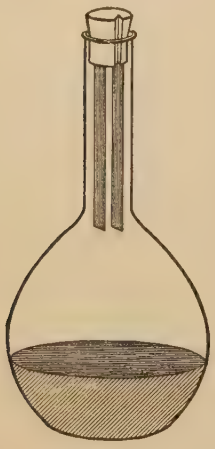


Fig. 1.

Moisten one strip with silver nitrate and the other with lead acetate solution.[1] Warm gently upon the water-bath (40–50°)[2] and allow to stand for some time protected from light. If the silver but not the lead paper is blackened, yellow phosphorus may be present. If both papers are blackened, hydrogen sulphide is also present. In the latter case, yellow phosphorus may be present with hydrogen sulphide. In absence of hydrogen sulphide, blackening of the silver paper is not final proof of yellow phosphorus, for any volatile organic substance having reducing properties, such as formaldehyde (H.CHO), or formic acid (H.COOH), may give the same result.

Scherer's test is of value in proving absence rather than presence of yellow phosphorus. It is a good preliminary test, as it excludes phosphorus if the silver paper is unchanged.

Distillation

Place a portion of finely divided and thoroughly mixed material in a large round-bottom flask and add enough distilled water for free distillation. Then add tartaric acid solution drop by drop until the mixture is acid after thorough shaking. In examining animal material, such as the stomach or intestines and contents, or

[1] To prepare a sensitive "lead paper," use an alkaline lead oxide solution instead of lead acetate. This is obtained by mixing a solution of a lead salt with excess of sodium hydroxide solution.

[2] Temperatures in this book are expressed in Centigrade degrees. Tr.

organs, such as liver, spleen, kidneys, or brain, it is often unnecessary to add much water because usually enough is present. First chop the material in a wooden tray with a steel knife. In a medico-legal case the tray should be new. A meat-machine that has been carefully cleaned may be used. Thin the material with a little distilled water, acidify with dilute tartaric or sulphuric acid and finally distil.

If Scherer's test is positive, begin distilling with the Mitscherlich apparatus; but if negative, distil in the usual way with a Liebig condenser.

Mitscherlich[1] Method of Detecting Phosphorus

The principle of this method is that yellow phosphorus volatilizes with steam and becomes luminous in contact with air. Phosphorescence is best seen in a dark room.

Procedure.—Arrange the apparatus as shown in Fig. 2. Support the condenser in a vertical position and connect the upper end with the flask by a glass tube about 8 mm. internal diameter. This tube has two right-angle bends and each end passes through a cork. Have condenser and tube scrupulously clean to avoid interference with phosphorescence.

Have the flask at most not more than a third full. This precaution is necessary because many materials, containing protein substances, such as albumin, albumose, etc., and starchy matter, when distilled in aqueous solution, cause more or less foaming that may carry over solid matter into the receiver. Use as the receiver an Erlenmeyer flask, containing a little distilled water (3–5 cc.), into which the end of the condenser dips. This precaution prevents loss of easily volatile substances such as hydrocyanic acid, chloroform and alcohol. Heat the flask upon a wire gauze of fine mesh, asbestos plate, or sand-bath, and bring the contents to boiling by raising the temperature gradually. There is some danger of burning or carbonizing organic matter on the bottom of the flask, if heat is applied too strongly or rapidly. When boiling begins, make the room as dark as possible and watch for phosphorescence in the tube and condenser. It usually appears as a luminous ring or band in the upper part of the condenser. When this is distinctly visible, presence of yellow phosphorus is established. Phosphorescence

[1] E. Mitscherlich: Method of Detecting Phosphorus in Poisoning Cases. Journ. f. prakt. Chem. 66 (1855), 238.

during distillation with steam is very characteristic of yellow phosphorus and frequently is the only positive test for this element.[1] In absence of interfering substances, the delicacy of the Mitscherlich test is very great. Hilger found that 0.06–0.3 mg. of phosphorus in

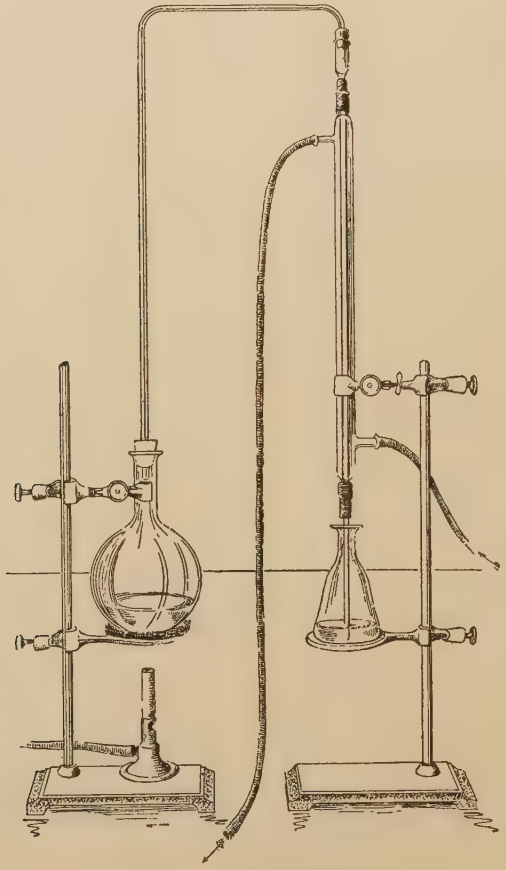


FIG. 2.—Mitscherlich apparatus.

200 cc. of water could be distinctly recognized and Fresenius observed that phosphorescence from 1 mg. of phosphorus lasted

[1] During distillation in the Mitscherlich apparatus, phosphorus sesquisulphide, P_4S_3, gives rise to phosphorescence like that produced by yellow phosphorus. If this phosphorus compound is present in the material, yellow phosphorus can be detected only with the electroscope by the method of Schenck and Scharff described in Chapter V (see page 476).

for half an hour. Obviously duration of phosphorescence depends upon rapidity of distillation.

Phosphorescence is a process of oxidation by which phosphorus vapor is changed to phosphorous acid. Should it not appear immediately, distillation must be continued for some time, since certain substances such as ethyl or methyl alcohol, ether, turpentine, acetic ether, and benzine, as well as many ethereal oils either prevent phosphorescence entirely or seriously retard it. Carbolic acid, creosote, chloroform, chloral hydrate, as well as hydrogen sulphide, may completely prevent phosphorescence. When yellow phosphorus is present, phosphorescence may also be prevented by substances that can convert phosphorus into non-volatile compounds. All oxidizing agents, and particularly salts of mercury,[1] copper and silver, are capable of acting in this manner. By this action, phosphorus is converted into oxygen or metallic compounds. Mercuric chloride and other mercury compounds can entirely prevent phosphorescence. Possibly mercuric chloride carried over by steam is reduced to metallic mercury by phosphorus vapor. In that case the metal should appear in the distillate. The fact that both metallic mercury and phosphoric acid can be detected in the distillate favors the supposition that action takes place between phosphorus vapor and mercuric chloride. In presence of those volatile substances that do not prevent volatilization of phosphorus but do interfere with the appearance of phosphorescence, the latter often will appear, though not always, when these interfering substances have been removed by distillation, provided their quantity is not too large and that of phosphorus too small. Distillation in such cases should be of long duration. By a modification of the Mitscherlich apparatus, Habermann and Oesterreicher were able to observe phosphorescence even in presence of ethyl alcohol. Where the steam entered the condenser they introduced a dropping-funnel containing cold water. The instant steam reached the condenser they injected a spray of water. Other substances that vaporize, such as turpentine and volatile phenols, permanently prevent appearance of phosphorescence. This is an important matter in view of the fact that resinified turpentine is used as an antidote for phosphorus poisoning and volatile phenols appear in the putrefaction

[1] K. Polstorff and J. Mensching: Test for Phosphorus by the Mitscherlich Method in Presence of Chlorides of Mercury. Ber. d. Deutsch. chem. Ges. 19 (1886), 1763.

of proteins. In presence of elementary phosphorus the distillate has the odor of this element and sometimes contains globules of phosphorus together with phosphorous acid.

In any event, even when phosphorescence has not been observed, convert phosphorus that may be present as well as phosphorous acid into phosphoric acid. This may be done by evaporating a considerable portion of the distillate in a porcelain dish upon the water-bath in presence of an excess of saturated chlorine water, strong bromine water, or a little fuming nitric acid. Dissolve the residue in a little water, divide the solution into two equal portions, and make the two following tests for phosphoric acid:

1. **Ammonium Molybdate Test.**—Acidify the solution with a few drops of concentrated nitric acid. Add an equal volume of ammonium molybdate solution and warm to about 40°. Phosphoric acid precipitates yellow ammonium phospho-molybdate.

FIG. 3.—Ammonium magnesium phosphate crystals.

2. **Ammonium Magnesium Phosphate Test.**—Add magnesia mixture[1] to the second portion. Phosphoric acid gives a white crystalline precipitate of ammonium magnesium phosphate, $(H_4N)MgPO_4.6H_2O$. Vigorous shaking favors precipitation. When only traces of phosphoric acid are present, long standing is necessary before the precipitate appears. Always examine the precipitate with the microscope. It should consist of well-formed crystals or at least be crystalline. These crystals are transparent, acicular prisms (Fig. 3).

Notes upon Detection of Phosphorus by Mitscherlich Method

The author regards it of the utmost importance to test for phosphoric acid in the distillate following oxidation in the manner described, especially when yellow phosphorus, owing to the defects of the Mitscherlich method mentioned above, cannot be recognized by the eye. When the distillate prepared as directed gives a positive test for phosphoric acid, the material contains yellow phosphorus,

[1] Magnesia mixture is a clear solution prepared by mixing equal volumes of magnesium chloride, ammonium chloride and ammonium hydroxide (about 10 per cent.) solutions. It contains the readily soluble double chloride of ammonium and magnesium which is not decomposed by ammonium hydroxide. This reagent is prepared as needed and should be perfectly clear and colorless.

provided phosphorus sesquisulphide is not present. When phosphorescence is not observed, the distillate obtained by the Mitscherlich method may also be shaken with silver nitrate solution. If a black precipitate appears, it can be filtered off at once, washed and examined for phosphorus in the Dusart-Blondlot apparatus (see page 8).

In all cases the author does not recommend testing the Mitscherlich distillate for phosphorous acid with silver nitrate and mercuric chloride, for distillates from cadaveric material quite often contain such substances as hydrogen sulphide, aldehyde-like bodies, or formic acid (a normal constituent of human urine). They give the same reactions with these two reagents that phosphorous acid does and consequently can simulate the presence of the latter. Slightly characteristic reactions of this description in the hands of not over-critical observers lead only to harm. At the same time such tests are merely a waste of expensive material.

The smallest quantity of yellow phosphorus that can be detected with certainty by the Mitscherlich method is 0.06 mg. When 200 cc. of water containing 0.3 mg. of phosphorus were distilled, there was brilliant phosphorescence for 5 minutes (Nattermann and Hilger). The degree of dilution seems to have no effect upon the result, at least not within limits occurring in practice. This also applies to hydrogen sulphide which is always given off when cadaveric material that has begun to putrefy is acidified and heated. When an acid aqueous solution is distilled in the Mitscherlich apparatus, the flask residue always contains phosphoric (H_3PO_4), phosphorous (H_3PO_3), and hypophosphorous (H_3PO_2) acids and red phosphorus. Distillation of a solution of 0.0644 gram of phosphorus gave only 71.33 per cent. in the distillate. The residue contained:

Phosphorus as phosphoric acid (H_3PO_4).....	18.93 per cent.
Phosphorus as phosphorous acid (H_3PO_3).....	2.15 per cent.
Phosphorus as hypophosphorous acid (H_3PO_2)...	4.27 per cent.
Phosphorus as red phosphorus.....	2.98 per cent.
	28.33 per cent.

A. Fischer[1] states that substances interfering more or less with the detection of phosphorus by the Mitscherlich method are usually less troublesome if Hilger and Nattermann's procedure is adopted (see page 18). The essential feature of this procedure consists in allowing steam charged with phosphorus to pass into the air from time to time, or in admitting air into the apparatus.

Phosphorus Sesquisulphide, P_4S_3.—In the manufacture of matches that will light anywhere, this compound is recommended as a substitute for white phosphorus which is forbidden in Germany. When distilled from an acid aqueous solution, it produces the same phosphorescence exhibited by toxic yellow phosphorus. For this reason the Mitscherlich method of detecting phosphorus cannot be regarded as free from objection, unless absence of phosphorus sesquisulphide has been positively established.

In the examination of the distillate, obtained by distilling a given material in a current of carbon dioxide according to the Scherer-Fresenius-Neubauer method,

[1] A Fischer: Contributions to the Detection of Phosphorus. Pflüger's Arch. 97 (1903), 578.

phosphorus sesquisulphide also interferes with the detection of phosphorus by the Dusart-Blondlot method, since in this case it is capable of simulating toxic elementary phosphorus. The physical method of R. Schenck and Scharff[1] is the only means of determining whether the given material contains elementary phosphorus or phosphorus sesquisulphide. Phosphorus vapor, probably because it undergoes oxidation even at ordinary temperature to phosphorus trioxide (P_2O_3), renders air a conductor of electricity, since it ionizes air and consequently discharges a charged electroscope. On the other hand, pure phosphorus sesquisulphide has no action whatever upon an electroscope at temperatures up to 50° and no considerable action up to 75°. Presence of only fractions of a milligram of white phosphorus renders air a conductor, for example, 0.004 mg. exerts a notable influence upon an electroscope. Temperatures of 35–55° are best adapted for these experiments.

Whether there is an actual difference between the physiological, that is, the toxic action of elementary white phosphorus and that of phosphorus sesquisulphide appears to be unknown. The work of Mörner, as well as that of Santesson and Malmgren, shows that a single dose of 0.5–1.0 gram of pure phosphorus sesquisulphide is apparently harmless. But if fed to animals for a longer time, it causes marked fatty degeneration of the liver as in phosphorus poisoning.

Detection of Phosphorus and Phosphorous Acid

(Dusart[2] and Blondlot[3])

Elementary phosphorus and its lower oxidation products, hypophosphorous acid (H_3PO_2) and phosphorous acid (H_3PO_3) are reduced to gaseous phosphine (PH_3) by nascent hydrogen:

$$H_3PO_2 + 4H = PH_3 + 2H_2O$$
$$H_3PO_3 + 6H = PH_3 + 3H_2O$$

Under the same conditions, ordinary or ortho-phosphoric acid (H_3PO_4) and its anhydro-acids, pyrophosphoric acid ($H_4P_2O_7$) and meta-phosphoric acid (HPO_3), are not reduced to phosphine. Phosphine and hydrogen charged with phosphorus vapor, when ignited, burn with a characteristic green flame (Dusart's reaction):

$$2PH_3 + 4O_2 = P_2O_5 + 3H_2O$$

This green flame coloration may be recognized especially well by holding in the flame a cold porcelain dish or, as in case of the "*chemi-*

[1] R. Schenck and E. Scharff: A Method for the Detection of Minute Quantities of White Phosphorus. Ber. d. Deutsch. chem. Ges. 39 (1916), 1522.

[2] Dusart: Note on the Detection of Phosphorus. Compt. rend. 43 (1856), 1126.

[3] Blondlot: Toxicological Detection of Phosphorus by the Flame Coloration. Compt rend. 52 (1861), 1197.

cal harmonica," over the flame a rather wide glass tube which is then lighted green its entire length. This test is sufficiently delicate to be distinctly visible even in a dilution of 1 milligram of phosphorus in 15 liters of hydrogen.

A toxicological analysis usually deals with the detection of traces of toxic phosphorus or of its first oxidation product, phosphorous acid. Hydrogen after acting in the nascent state upon the material is not directly examined for phosphorus but is first passed into dilute silver nitrate solution. Phosphine precipitates black silver phosphide (Ag_3P):

$$PH_3 + 3AgNO_3 = Ag_3P + 3HNO_3$$

Thus traces of yellow phosphorus, or of phosphorous acid, that may be in the cadaveric material, are concentrated in the black silver precipitate from which nascent hydrogen, prepared from phosphorus-free zinc and dilute sulphuric acid, will liberate phosphine:

$$Ag_3P + 3H = 3Ag + PH_3$$

When ignited, this will then give the Dusart reaction.

The method discovered by Dusart and introduced by Blondlot into forensic chemistry for the detection of minute quantities of phosphorus is based upon these facts.

Silver phosphide is not suitable for the quantitative estimation of phosphorus owing to its slight stability. In the moist condition, especially by the nitric acid resulting from its formation, it is oxidized to phosphoric acid and silver:

$$2Ag_3P + 5O + 3H_2O = 6Ag + 2H_3PO_4$$

Consequently the black precipitate formed by passing phosphine into silver nitrate solution always contains metallic silver together with silver phosphide.

If hydrogen produces a black or gray precipitate in a silver nitrate solution, phosphorus is not necessarily present, since hydrogen sulphide, arsine, stibine and various reducing organic compounds, such as formaldehyde and formic acid, also produce black precipitates. A black precipitate therefore should always be examined for phosphorus by the Dusart reaction. In the detection of yellow phosphorus and phosphorous acid, the Dusart-Blondlot method combines two distinct operations, namely:

1. Preparation of the silver phosphide precipitate.
2. Examination of this precipitate for phosphorus.

I. Preparation of Silver Phosphide

Thin the finely divided material, or the residue remaining in the distillation-flask from the Mitscherlich test, with water in a capacious flask having a doubly-bored stopper through which pass a dropping-funnel and a right-angle exit-tube. Connect the latter with a U-tube containing pieces of pumice-stone saturated with concentrated potassium hydroxide solution to absorb hydrogen sulphide. Attach to this U-tube the absorption-apparatus for phosphine. To insure complete absorption of phosphine by the silver nitrate solution, it is advisable to use a Liebig potash-apparatus employed in the analysis of organic substances, or a Péligot tube.[1] The absorption-apparatus is partly filled with 3 per cent. neutral silver nitrate solution. Granulated, phosphorus-free zinc is put into the evolution-flask before the material is introduced. Then run in pure dilute sulphuric acid (1:5), until there is a fairly active evolution of hydrogen. If the evolution of hydrogen subsides, more sulphuric acid should be added. Allow the action to continue for 6–8 hours or longer. If the material contains elementary phosphorus, or phosphorous acid, a black precipitate of silver phosphide will appear in the silver nitrate solution. Collect the latter upon a small ash-free paper, wash quickly with a little cold water, and examine for phosphorus as directed below in the Fresenius-Neubauer apparatus, or in that of Hilger-Nattermann, or by heating according to the method of Stich.

Phosphoric Acid Test.—If the precipitate is silver phosphide, the filtrate will contain phosphoric acid. To test for the latter, first add dilute hydrochloric acid to remove silver from the filtrate. Collect the silver chloride upon a paper previously well-washed with dilute nitric acid and water, and completely expel hydrochloric acid from the filtrate by evaporation upon the water-bath after adding nitric acid. Dissolve the residue in a little water and finally test for phosphoric acid by warming gently with ammonium molybdate solution. If a yellow precipitate appears, collect it upon a small filter, wash, and dissolve in a little ammonia. Add magnesia mixture to the clear solution and note whether a crystalline precipitate of ammonium magnesium phosphate appears (see page 6).

[1] J. Gadamer recommends for the absorption of phosphine two Liebig bulbs connected together, or two Hugershoff wash-bottles with flat glass spirals.

II. Examination of the Silver Precipitate for Phosphorus

In this test the silver precipitate obtained in one of the four following ways may be used:

(1) That obtained direct with silver nitrate in (1) by the action of nascent hydrogen upon the material.

(2) That formed when the distillate by the Mitscherlich method is shaken with silver nitrate.

(3) That obtained in the Fresenius-Neubauer procedure by heating the material in a current of carbon dioxide according to Scherer's method.

(4) That formed in distilling by Scherer's method as modified by Hilger and Nattermann.

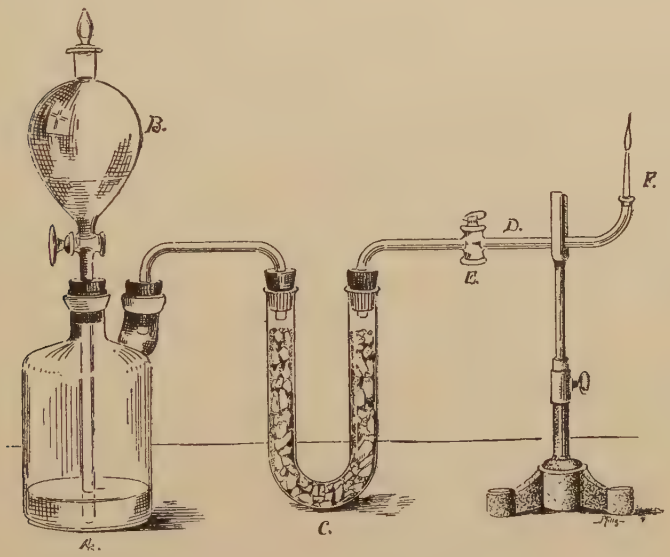


FIG. 4.—Fresenius-Neubauer apparatus.

(a) Fresenius-Neubauer[1] Apparatus

Apparatus.—This is shown in Fig. 4. Hydrogen is generated in flask (A) from pure phosphorus-free zinc and dilute sulphuric acid. Reservoir (B) serves to hold liquid from (A) when cock (E) is closed. U-tube (C) contains pieces of pumice-stone saturated with concentrated potassium hydroxide solution to absorb any hydrogen sulphide

[1] C. R. Fresenius: Qualitative Chemical Analysis, XVI Edition, page 521.

which interferes with the reaction. A tube of hard glass (D) is provided with a glass stop-cock (E) and a tip[1] (F) of platinum. A platinum tip is absolutely essential, for otherwise the flame instead of being colorless will always be yellow from sodium in the glass. The place where the platinum tip is fused into the glass should be cooled by wrapping cotton around the glass and keeping it moist.

Procedure.—Open (E) and allow hydrogen from (A) to pass through the apparatus for some time to expel air. Then close (E) and the liquid in (A) will rise into (B). Now open (E) just enough to allow hydrogen to burn with a small flame which should be colorless in the dark. If no trace of green is to be seen in the inner cone and a porcelain dish depressed upon the flame does not show an emerald-green coloration, hydrogen is phosphorus-free. It is well to repeat this test. To test for phosphorus in the precipitate obtained with silver nitrate, wash it with the paper into (B) with a little water, making sure that finally all is brought into (A). Then repeat the test in the same manner, that is, close cock (E) and open it when all the liquid in (A) has risen into (B). Now ignite the hydrogen and examine the flame in the dark as above. If the precipitate contains a trace of silver phosphide, the inner cone will be green and a porcelain dish depressed upon the flame will show an emerald-green coloration. Adjust cock (E) so that the hydrogen flame is small and its color may be observed for some time.

(b) Hilger-Nattermann[2] Apparatus

Reduction takes place in a 100 cc. flask closed by a rubber stopper with three holes, two of which are for right-angle tubes just passing through the stopper. Hydrogen from a Kipp generator is first washed through water and then enters the flask by one tube and leaves by the other. Attach to the latter a U-tube filled with pieces of pumice-stone saturated with concentrated sodium hydroxide solution to absorb hydrogen sulphide. Connect the other end of the U-tube with a hard glass tube tipped with platinum.[3] Through

[1] Fresenius and Neubauer use a screw pinch-cock instead of a gas-cock but by means of a short rubber connector they interpose an ordinary cock between gas-flask (A) and U-tube (C).

[2] H. Nattermann and A. Hilger: Detection of Phosphorus in Forensic-Chemical Investigations. Forschungsbericht über Lebensmittel und ihre Beziehungen zur Hygiene, 4 (1897), 241–258.

[3] Hilger and Nattermann use a platinum-tipped blow-pipe instead of a glass tube tipped with the same metal. Cotton, which is kept moist and acts as a cooler, is wrapped around the blow-pipe below the tip.

the third opening in the stopper a thistle-tube extends to the bottom of the flask. Cut the paper containing the precipitate into small pieces and place in the flask which also contains a few pieces of phosphorus-free zinc and enough water to seal the thistle-tube. Light the hydrogen after it has passed through the apparatus for some time and been found free from air by the usual test. Seen in the dark the flame should be entirely colorless and burn without a green cone or a greenish glow.[1] Hilger and Nattermann advise a spectroscopic examination of the flame to determine the purity of the zinc. Pure zinc gives a hydrogen spectrum showing only an orange-colored line in place of the yellow sodium line. The minutest

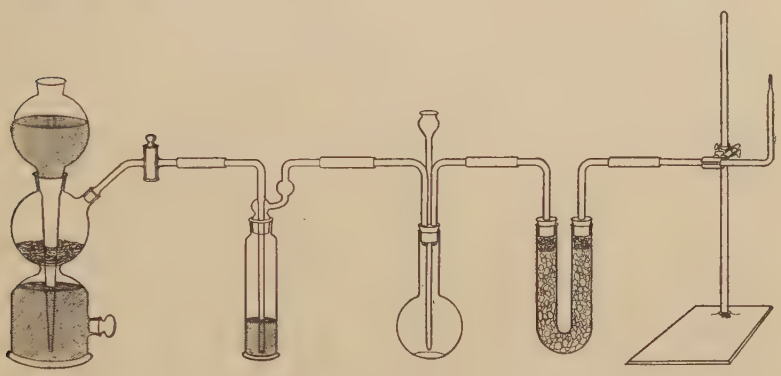


FIG. 5.—Hilger-Nattermann apparatus.

trace of phosphorus will give three green lines lying to the right of line D. The color of two of these lines is more pronounced than that of the third. Having thus tested the purity of the zinc and sulphuric acid, pour a few cc. of dilute sulphuric acid (1:5) through the thistle-tube into the flask containing zinc and the silver precipitate. If the latter contains phosphorus, the flame will show, though not always at once, a green coloration that should be examined with the spectroscope.

According to Dalmon, the Dusart reaction can be made in the manner of the "*chemical harmonica*" by holding a glass tube over the hydrogen flame and pressing the latter on the inner wall of the glass tube. If phosphorus is present, a beautiful green luminescence will extend throughout the tube and phosphorescent light waves

[1] Zinc entirely free from phosphorus which will stand this test is difficult to obtain.

will appear together with small detonations. If the flame is compressed by lowering the glass tube, a dark blue coloration will appear.

(c) Decomposition of the Silver Phosphide Precipitate by Heating (Stich[1])

Stich decomposes a black silver precipitate not by nascent hydrogen prepared from zinc and sulphuric acid, but by carefully applying gentle heat to the dry precipitate in a current of hydrogen. In this manner phosphine is also liberated from silver phosphide. Stich has hardly ever succeeded in obtaining zinc that is absolutely phosphorus-free. The author has experienced the same difficulty. Various commercial samples of zinc said to be chemically pure, as well as an electrolytic zinc, gave hydrogen that did not exhibit an emerald-green cone in the Dusart-Blondlot tests but thin blue-green streaks. The latter very decidedly interfered with the recognition of phosphorus in the Dusart-Blondlot test, if they did not make it quite impossible. For this reason Stich first passes hydrogen through glass-wool moistened with 3 per cent. silver nitrate solution, thereby getting a hydrogen flame that is absolutely colorless.

The black silver precipitate is collected upon a plug of pure asbestos in an Allihn filter-tube, washed and dried with gentle heat or in a vacuum-desiccator. The hydrogen obtained from zinc and dilute sulphuric acid in a Kipp generator is first passed through 3 per cent. silver nitrate solution to render it phosphorus-free and then dried with concentrated sulphuric acid. On the other side the Allihn filter-tube, connected with a U-tube containing pieces of pumice-stone saturated with potassium hydroxide solution, terminates in a platinum-tipped glass tube (Fig. 6). Hydrogen is first passed through the apparatus to displace air, then lighted and the suspected silver phosphide precipitate in the Allihn tube heated with a very small flame. If the black precipitate contains silver phosphide, the latter is decomposed with formation of phosphine producing a green cone in the flame. Iron obtained by igniting ferrous oxalate in a current of hydrogen may also be substituted for absolutely pure zinc.

Notes.—According to Nattermann and Hilger, 1/10 or at most 1/5 of the phosphorous acid is reduced to phosphine in the Dusart-Blondlot method. The

[1] C. Stich: Formation of Gaseous Compounds of Phosphorus in Putrefaction. Mitteilungen a. d. analyt. Laborat. d. Krankenhausapotheke zu Leipzig.

delicacy of the Dusart-Blondlot method is very great. By this flame test even 1/10,000 of a milligram of phosphorus is said to be recognizable (Nattermann and Hilger). Oxidation of phosphorus in a mixture of organic substances of varying composition such as food residues, or in any organic material in process of decomposition, sometimes proceeds rather slowly. Putrefactive processes in particular more or less prevent oxidation of phosphorus. Even after six months 3 milligrams of phosphorus were detected in organic material that had entered into putrefaction (Nattermann and Hilger).

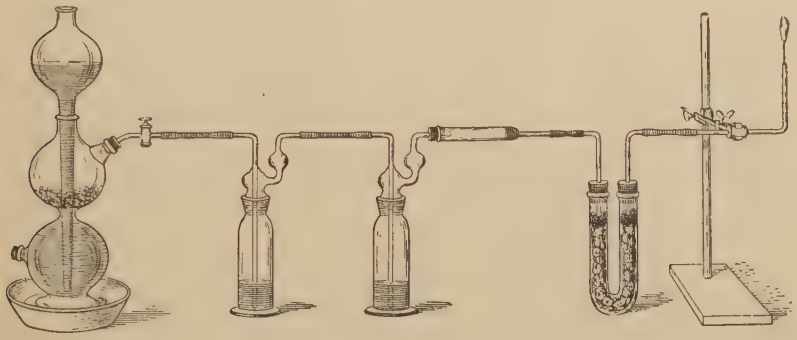


FIG. 6.—Apparatus of Stich.

Absorption of Phosphine by Cuprous Chloride

(Hilger and Nattermann)

To absorb phosphine, Hilger and Nattermann use a hydrochloric acid solution of cuprous chloride, first recommended by Ribau[1] for estimating PH_3 in gas mixtures. A molecular compound of the two substances is formed but at 70° is again resolved into its components.

Dissolve 4 grams of pure cuprous chloride in 20 cc. of hydrochloric acid (sp. gr. 1.19 = 37.5 per cent.) and add 30 cc. of 12 per cent. potassium hydroxide solution. After about 4 hours decant the clear solution from the crystals and put 20 cc. of this solution in each of two wash-bottles that are connected. Between the flask containing the material to be examined together with zinc and dilute sulphuric acid and one of the two wash-bottles containing the cuprous chloride solution is introduced an empty flask. The tubes by which the gas enters and leaves the latter end just below the stopper, to prevent cuprous chloride solution from getting back into the evolution-flask. After the action has proceeded for several days (14 days according to Hilger and Nattermann), pour the hydrochloric acid-cuprous chloride solution from the two wash-bottles into a boiling-flask provided with a doubly bored stopper through which two right-angle tubes pass. These tubes end just below the stopper. One serves as the entrance-tube for hydrogen prepared in a Kipp generator from pure zinc and dilute sulphuric acid and washed through water. The other as the exit-tube is connected with a U-tube containing pieces of pumice-stone saturated with potassium hydroxide

[1] Ribau: Comptes rend. 88 (1879), 11.

solution. The latter is attached to a glass tube tipped with platinum. As soon as hydrogen has completely displaced the air in the apparatus, it is lighted and tested for phosphorus. If found free from phosphorus, the cuprous chloride solution is warmed upon the water-bath. At about 70° phosphine begins to come off, mixed with hydrogen, and imparts the characteristic green coloration to the flame.

Detection and Estimation of Phosphorus by Combined Methods of Scherer and Dusart-Blondlot in Fresenius-Neubauer Procedure[1]

This method is based upon the volatility of yellow phosphorus in a stream of carbon dioxide from material acidified with sulphuric acid, the precipitation by silver nitrate of phosphorus carried over in the distillate, and its detection in the black precipitate formed by means of the Dusart reaction. This method is very useful for the quantitative estimation of phosphorus in any material whatever.

Procedure.—Distillation is conveniently carried out in the apparatus shown below (Fig. 7). Comminute the material as finely as

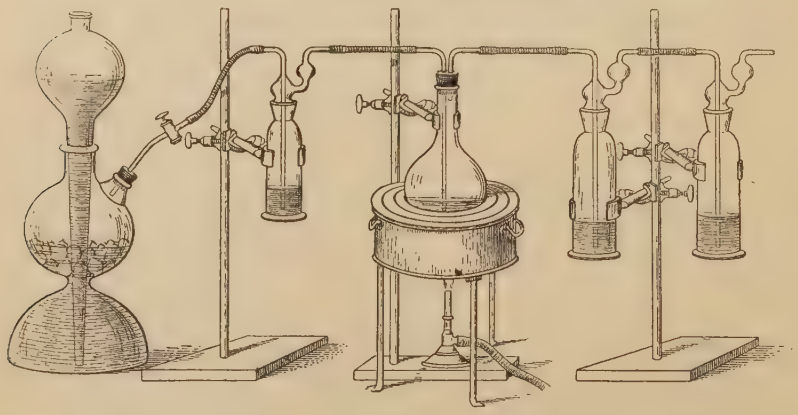


FIG. 7.—Fresenius-Neubauer apparatus for quantitative estimation of phosphorus.

possible, introduce into a capacious but not too large flask, if necessary with addition of a little water, and acidify with dilute sulphuric acid. The flask is provided with a doubly bored stopper through one opening of which a right-angle tube, extending nearly to the bottom, serves for the introduction of gas. The right-angle tube passing through the other opening ends just below the stopper and is in connection with two absorption-bottles one-third full of 3 per

[1] Zeitschrift f. analyt. Chem. 1 (1862), 129.

cent. silver nitrate solution. A Péligot-tube may also be used to hold the silver nitrate solution. Carbon dioxide from a Kipp generator is washed through water and passed through the apparatus to remove air as completely as possible which may require 20–30 minutes. Then, while keeping up the stream of carbon dioxide, warm the flask to 60°, or at most to 70°, so that too much water vapor is not carried over into the receivers. In a qualitative test for phosphorus it is sufficient to pass carbon dioxide through for 2–3 hours. In a quantitative experiment carbon dioxide should be passed through until the vapors that issue when the apparatus is opened at the place where the gas-mixture enters the absorption-bottles no longer exhibit phosphorescence in a darkened room. As a rule, if the current of carbon dioxide is rapid, 50 milligrams of phosphorus will pass over in 8–10 hours. For a qualitative test collect the black precipitate upon a small filter washed with dilute nitric acid and water, rinse with a little water and examine for phosphorus in the Dusart-Blondlot apparatus or, in case the precipitate is in an asbestos filter-tube, by the method of Stich.

The filtrate from the precipitate of silver phosphide always contains phosphoric acid. To detect it, remove excess of silver with hydrochloric acid, evaporate the filtrate upon the water-bath, and test the solution of the residue in a little dilute nitric acid by warming it at about 40° with ammonium molybdate solution. For the quantitative estimation of phosphorus, evaporate the contents of the absorption-bottles upon the water-bath with an excess of aqua regia, extract the residue with a little hot water containing a few drops of dilute nitric acid, filter, saturate the filtrate evaporated to about 10 cc. with ammonia, precipitate phosphoric acid with clear magnesia mixture, and finally weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

Notes.—The Fresenius-Neubauer procedure is especially recommended, if the material contains substances that interfere with phosphorescence in the Mitscherlich apparatus. In such a case the distillate obtained by the Mitscherlich method may be shaken with silver nitrate solution, or the distillate may be caught in silver nitrate solution, and a black precipitate tested for phosphorus by the Dusart-Blondlot or Stich method. In quantitative experiments the author has repeatedly determined phosphorus in the black precipitate and phosphoric acid in the filtrate that came from the latter. As a result it has been found that 30–40 per cent. of the total phosphorus was present as phosphoric acid in the filtrate from the phosphorus precipitate.

Detection and Estimation of Phosphorus by Mitscherlich-Scherer Method Modified by Nattermann and Hilger[1]

By a convenient modification Nattermann and Hilger have combined the Mitscherlich method of detecting phosphorus with that of Scherer, thus making it possible to produce the very characteristic phosphorescence as well as to obtain as large a quantity of phosphorus as possible in the distillate and to distil in a current of carbon dioxide. This procedure is always to be recommended, if a qualitative test for phosphorus is to be made in conjunction with a quantitative estimation.

Procedure.—Distillation is conducted in the apparatus of Nattermann and Hilger shown below (Fig. 8). In order to produce phosphorescence in the horizontal part of the Mitscherlich air-cooler not far from the place where it becomes perpendicular and enters the evolution-flask, Nattermann and Hilger introduce a 30 cm. glass tube provided with a rubber connector and screw-clamp or glass stop-cock. The receivers are an Erlenmeyer flask tightly connected with the condenser and a glass cylinder. Both contain 3 per cent. silver nitrate solution. Gadamer uses a Péligot-tube instead of the cylinder. These receivers are connected with a suction-pump. As soon as the apparatus is ready, pass through the flask, containing the comminuted material acidified with tartaric acid or dilute sulphuric acid, for at least half an hour a gentle stream of carbon dioxide washed through water so that the entire apparatus is free from air. Then gradually heat the flask containing the material in an air-bath or paraffine-bath all the while maintaining the stream of carbon dioxide. As soon as the doubly bent right-angle tube connecting the flask and condenser begins to get warm, start the suction-pump and at once admit air by means of the screw-clamp or glass stop-cock. If only traces of phosphorus vapor are present in the distillation-tube and condenser, brilliant phosphorescence will appear as soon as they meet the air that is drawn in. Phosphorescence is said to appear even in presence of those volatile substances that prevent it entirely in the Mitscherlich apparatus (A. Fischer). Having observed phosphorescence, close the clamp or stop-cock and continue distillation until a further test with air

[1] H. Nattermann and A. Hilger: Detection of Phosphorus in Forensic-Chemical Investigations. Forschungsberichte über Lebensmittel und ihre Bez. z. Hygiene 4 (1897), 241.

is negative. To determine phosphorus quantitatively, evaporate the contents of the receivers, that is, solutions and precipitates, in a porcelain dish upon the water-bath with a little aqua regia. Dissolve the residue in water containing hydrochloric acid, filter from silver chloride, precipitate phosphoric acid in the filtrate with clear magnesia mixture, and finally weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

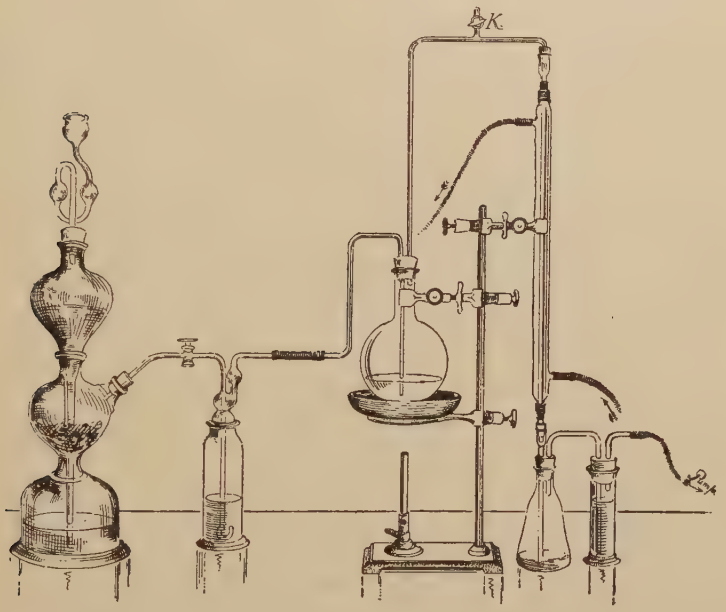


FIG. 8.—Hilger-Nattermann apparatus for detecting and quantitatively estimating phosphorus.

Notes.—Obviously the black silver precipitate may be used for the Dusart reaction, in case this test is necessary. By their procedure Nattermann and Hilger obtained in the distillate 92.4–95.1 per cent. of the phosphorus present. In accuracy it is hardly inferior to Scherer's procedure and gives moreover the very characteristic phosphorescence.

Are Volatile Phosphorus Compounds Formed in Putrefaction and by Action of Nascent Hydrogen upon Normal Phosphorus Compounds in the Animal Organism?

Opinions of different investigators, who have turned their attention to this question, are not in accord.

According to Stich,[1] gases containing phosphorus are formed in certain putrefactive processes of animal and plant substances. What the nature of these gases is cannot be determined because they are formed in such small quantity. The putrefactive bacteria used were in part bacteria Coli communis and in part mixed cultures, that is, from putrefying pancreatic juice. The culture-media consisted of alkaline solutions (1 per cent. each) of glucose and protein, that is, peptone, casein, sodium caseinate, nuclein, lecithin from the yolk of egg and protagon. Stich also subjected a number of animal and plant substances, especially internal organs in the fresh condition, to putrefaction at 37°. The gases formed in the putrefaction of protein substances were free from phosphorus and the residue from putrefaction gave none of the reactions that indicate the lower stages of oxidation of phosphorus. But it is true that phosphoric acid was detected in the residue from the putrefaction of casein, nuclein, lecithin and protagon. These substances also gave phosphoric acid as a result of the action of nascent hydrogen. According to Stich, gases containing phosphorus are obtained as a result of the action of nascent hydrogen from putrefying fish, brain, potatoes, and also when these are fresh. Upon the basis of his experimental results Stich maintains that only the detection of elementary phosphorus should be accepted as indisputable proof of phosphorus poisoning. A. Fischer[2] was unable to confirm the results of Stich's experiments wherein gases containing phosphorus were obtained from putrefying potatoes as well as from fresh potatoes as a result of the action of nascent hydrogen. Nor was he able to establish the fact that such gases were given off from putrefying brain. Previously Z. Halász[3] had obtained results identical with those of Fischer. He examined two kinds of animal material by the Dusart-Blondlot method. First, he tested normal brains (man, calf, hog); second, the brain and other organs of rabbits that had been given poisonous doses of phosphorus by the mouth and subcutaneously. He examined these organs when fresh and also from week to week after more or less pronounced putrefaction had set in, but could not detect phosphorus in the brain in a single instance. These experiments disprove the earlier idea that phosphorus normally present in the brain is so changed during putrefaction that it can be detected by the Dusart-Blondlot method. He also failed to detect phosphorus or phosphine in the brain of rabbits poisoned by this element, though he found it in other organs, such as stomach and intestines, and in those rich in blood, such as liver, lungs and kidneys. He could always detect small or large quantities of phosphorus in any organ which this element had directly reached, or by which it had been indirectly absorbed. Halász concludes from his experiments that if any volatile phosphorus compound is formed in the brain during putrefaction, it is not volatile with steam but with alcohol and does not give the Dusart-Blondlot reaction. This amounts to saying that such a compound cannot be detected by the forensic-chemical method. On the basis of these experiments Halász holds

[1] C. Stich: Formation of Gaseous Compounds of Phosphorus in Putrefaction. Mitt. a. d. analyt. Laborat. zu Leipzig 22 (1900).

[2] A Fischer: Contributions to the Detection of Phosphorus. Pflüger's Archiv. der Physiologie 97 (1903), 578.

[3] Z. Halász: Is the Blondlot-Dusart Method reliable in Forensic-Chemical Cases? Zeitschr. f. anorg. Chemie 26 (1900), 438.

that the Dusart-Blondlot method of detecting phosphorus is just as reliable for forensic purposes as that of Mitscherlich.

Duration of Yellow Phosphorus in the Cadaver

Yellow phosphorus can be detected after a considerable time in any organic matter whatever, even when it has reached a high state of putrefaction. Processes of putrefaction and decay prevent or at least retard the oxidation of phosphorus. In the cadaver phosphorus is also only slowly oxidized. This fact may be explained when it is recalled that atmospheric oxygen is necessary for the oxidation of phosphorus and that during putrefaction reducing gases that protect phosphorus from oxidation are formed. For this reason after poisoning phosphorus frequently can be detected as such in the cadaver after a rather long time. Dragendorff detected phosphorus in an exhumed body several weeks after death. Elvers found free phosphorus in a human body eight weeks after death. Bischoff found phosphorus after six months and Poleck[1] even earlier had proved the presence of traces of free phosphorus in a cadaver three months after poisoning had occurred. Recently Alpers[2] found even 13.9 mg. of elementary phosphorus in the gastro-intestinal contents of a woman who had died as a result of acute phosphorus poisoning and whose body was not exhumed until four weeks after death. In this investigation Alpers observed that phosphorus distils over very slowly even in a rapid current of steam. Distillation of phosphorus did not come to an end until after about 8 hours.

Absorption and Toxic Action of Phosphorus

Absorption of phosphorus takes place from every mucous surface and wound. The form in which phosphorus is absorbed is a question that cannot be answered with certainty. In view of the powerful, specific toxic action of phosphorus, however, it must be assumed that it gets into the circulation dissolved in water, bile or fat, or in the form of vapor. This hypothesis as to the absorption of phosphorus in the elementary condition finds support in the fact that after poisoning by phosphorus it can be detected as such in the liver, in the blood and in expired air. In this connection mention should be made of the retention of phosphorus as such unchanged in the blood for hours and even for days. Phosphorus dissolved in oils is held at ordinary temperature by arterial as well as by venous blood, but not by serum. Phosphorus can be detected both chemically and spectroscopically in expired air from animals poisoned by phosphorus. In case of poisoning by large intra-arterial doses of phosphorus, the expired air is highly ionized. Consequently in such cases, elementary phosphorus gets from the blood into the alveolar wall where it is oxidized (Schmidt[3]).

Evidences of phosphorus poisoning in many cases do not appear for some time, occasionally not for several hours after the phosphorus has been taken, in very

[1] Th. Poleck: Detection of Phosphorus Poisoning in a Cadaver three months after Death. Archiv. d. Pharmazie 225 (1887), 189.

[2] K. Alpers: Detection of Elementary Phosphorus in a Cadaver four weeks after Death. Pharm. Ztg. 58 (1913), 127.

[3] H. Schmidt: A Contribution to our Knowledge of Phosphorated Oils, and their Retention in the Organism, by means of the Electroscopic Detection of Phosphorus. Biochemische Zeitschr. 34 (1911), 280.

rare instances not for one or two days. In the gastric region there is a burning sensation with pains and, preceded by belching and choking, there ensues vomiting of masses that are luminous in the dark and smell of phosphorus. The exhaled air has a more or less strong smell of phosphorus. Usually there is severe burning in the throat and diarrhoea in about 30 per cent. of the cases. The liver begins to swell and as it increases in size, the face usually on the second or third day after the poison has been taken shows the characteristic icteric discoloration which may confine itself to that area or extend over the entire body. Additional occurrences in acute phosphorus poisoning are: nose-bleed, bleeding from the intestines and in women from the genitals, muscular paralysis, ringing in the ears, even deafness, swimming of objects before the eyes, and dullness of vision. The pulse is irregular; the patients become stupefied, delirious, quiet and end in a state of coma (Lewin).

The average lethal dose of phosphorus is from 0.1–0.2 gram, although a quantity as small as 0.05 gram has given rise to fatal poisoning. On the other hand, much larger doses of 0.3–0.5 gram of phosphorus, especially if taken in one piece, have produced only milder symptoms of intoxication. As a rule death ensues within six days and in most cases on the second or third day; in isolated cases 7.9 hours after the poison was taken.

In a case of suspected phosphorus poisoning, gastric and intestinal contents, blood, and organs rich in blood, such as the liver, should be examined. Vomitus is also an important material for examination.

Metabolism in Phosphorus Poisoning

In very small daily doses of 0.5–1 milligram, phosphorus exerts in man an increasing influence upon metabolism, that is, upon growth and formation of new tissue. Physicians have come to this conclusion from observing clinically the favorable influence upon the condition of nutrition as a whole and the unusual increase in weight of children treated with phosphorus for rachitis. (Kassowitz, Hagenbach 1884.) The action of phosphorus in man in the beginning causes an increase in the number of red blood-corpuscles and also exerts a favorable influence upon bone-formation. In larger quantities phosphorus has a very poisonous action upon the processes of metabolism. Under its influence a marked increase in the breaking down of tissue ensues together with accompanying disturbances of the processes of oxidation, synthesis and decomposition. In phosphorus poisoning the normal course of chemical metabolism undergoes complete derangement. The consumption of oxygen as well as the formation of carbon dioxide is diminished; less fat is decomposed but so much the more carbohydrate and protein; the latter, however, is only incompletely broken down so that products indicating partial decomposition, such as peptones, amino-acids and lactic acid are found in blood and urine.

In consequence of incomplete combustion of fat, it is deposited in the organs in large quantity. This is distinctly noticeable in the liver, heart, kidneys, partly also in the diaphragm and in the other muscles which collectively give more or less evidence of fatty degeneration. In the liver and heart it is probably also a question as to the transference of fat from other tissues; it may arise in part from the subcutaneous deposit.

The decomposition of protein, as stated above, shows a marked increase in phosphorus poisoning. The protein of organs is broken down more extensively but not as completely as in normal protein metabolism. Porges and Pribram[1] investigated protein exchange in the liver of animals poisoned with phosphorus and found that the phosphorus liver just removed from the animal was relatively poorer in protein than the normal liver. Autolytic cleavage of protein in the phosphorus liver as contrasted with autolysis in normal livers is materially increased. In phosphorus poisoning as in respiration in air very poor in oxygen decomposition of body tissues shows an increase with partial formation of such nitrogenous and non-nitrogenous products of metabolism as normally either are not formed at all in the organism or appear merely as intermediate products of oxidative animal metabolism. Decomposition of the protein molecule goes in part only as far as the amino-acids. Consequently in phosphorus poisoning the urine almost always contains leucine, $(CH_3)_2CH.CH_2.CH(NH_2).COOH$, tyrosine, $HO.C_6H_4.CH_2.CH(NH_2).COOH$ (1:4), and also sarco-lactic acid, $CH_3.CH(OH).COOH$. Acids that normally occur in the urine only in traces may be detected in much larger quantity in acute phosphorus poisoning. Such acids are p-oxyphenyl-acetic acid, $HO.C_6H_4.CH_2.COOH$ (1:4) and p-oxyphenyl-propionic acid, or hydropara-cumaric acid, $HO.C_6H_4.CH_2.CH_2.COOH$ (1:4). Cystine, $HOOC.CH(NH_2).CH_2.S\text{-}S.CH_2.CH(NH_2).COOH$, has also been repeatedly found in phosphorus urine.

In phosphorus poisoning there is a marked decrease in the urea-content of the urine but a decided increase in total nitrogen. A considerable part of the nitrogen, that is, 25 per cent. or more of the total nitrogen, appears to leave the body as ammonia, whereas the urine of adults usually contains from 2–5 per cent. of the total nitrogen as ammonia. This increase of ammonia may have some connection with the increase in formation of acid during phosphorus poisoning, since the ammonia serves to neutralize the acid formed.

Peptone-like substances, the presence of which is attributed to profound disturbance of metabolism, frequently appear in the urine in phosphorus poisoning. Various observers believe that there is no longer any doubt as to the appearance of genuine peptonuria. A glycosuria may also appear, or be easily induced by a diet rich in sugar. In accord with this observation is the fact that the liver of an animal poisoned by phosphorus is without the power to change glucose of the blood into glycogen and store up the latter. Since persons poisoned by phosphorus have icterus (jaundice), bile-pigment, or an abundance of urobilin, can almost always be detected in the urine.

The disturbances of metabolism evoked by phosphorus coincide in many respects with the anomalies that arise from deficiency of oxygen. Consequently it is possible that the cells of the body poisoned by phosphorus are less capable of utilizing normally the oxygen that is taken up. This is shown by the fact that the amounts of oxygen and carbon dioxide, which the organism respectively takes up and gives off, show a marked diminution during phosphorus poisoning. Only 48 per cent. of carbon dioxide, as compared with 100 per cent. under normal

[1] O. Porges and E. Pribram: Contribution to the Knowledge of the Chemical Processes in Phosphorus Poisoning. Archiv. f. exp. Pathol. u. Pharmak. 59 (1908), 20

conditions, may be eliminated. Although the phosphorus that gets into circulation does not reduce the oxyhaemoglobin of the blood, it increases its power of transferring oxygen to oxidizable substances. The free phosphorus in the animal organism is only very gradually oxidized to phosphorous acid and finally to phosphoric acid.

Urine secreted during phosphorus poisoning has a strong acid reaction, usually containing lactic acid, almost always protein or peptone-like substances, and the above mentioned amino-acids, as well as fat-cylinders, cell-detritus, free fat globules and blood-corpuscles.

Oxidation of Phosphorus, Luminescence and Electrical Conductivity of Phosphorus Air

Schenck, Mihr and Banthien,[1] as well as Scharff,[2] have thoroughly investigated the relations existing between oxidation of phosphorus, luminescence and electrical conductivity of phosphorus air and arrived at the following results. White phosphorus is first oxidized to phosphorus trioxide, P_4O_6, without the appearance of luminescence, the latter not appearing until further oxidation takes place. In moist oxygen the luminescence of phosphorus trioxide is strongly marked and very beautiful, whereas it fails to appear in absolutely dry oxygen. In this case is very distinctly shown the phenomenon of "intermittent luminescence" and its dependence upon the proportion of oxygen in the mixture. Since phosphorescence depends upon the presence of oxygen and water vapor, that is, upon moist air, and since it has further been uniformly shown that conductivity in presence of white phosphorus takes place only while processes of oxidation are going on, obviously all factors checking oxidation will prevent luminescence as well as the electrical conductivity of phosphorus air. Complete parallelism exists between the appearance of the oxidation luminescence and the ionization of phosphorus air, for these phenomena cease as soon as the vapor of oil of turpentine, alcohol, ethylene, mesitylene, ammonia, carbon disulphide, or illuminating gas is added to the air before it passes over the phosphorus.

Various oxidation phenomena of entirely different substances are quite similar to those of phosphorus. Sulphur, for example, at 200° undergoes slow oxidation and exhibits phosphorescence. At the same time there appears an oxidation product smelling like camphor and very irritating to the eyes (Haumann). Brom-acetylene, $CH{:}CBr$, which Nef regards as brom-acetylydene, $CHBr{:}C$, is said to behave very much like phosphorus. Water and alcohol dissolve it in small quantities; its solutions phosphoresce in the dark just as phosphorus does and exhibit a strong ozone reaction and a distinct ozone smell. In its physiological action brom-acetylene is said to be very similar to phosphorus. According to Schenck ozone, phosphorus pentoxide and its hydrates cannot be considered as exciters of electrical conductivity, but probably vapors of phosphorus trioxide have an ionizing action upon air. The action of the vapor of phosphorus trioxide

[1] R. Schenck, F. Mihr, H. Banthien: The Component of Phosphorus Air causing Electrical Conductivity. Ber. d. Deutsch. chem. Ges. 39 (1906), 1506.

[2] E. Scharff: Luminescence of Phosphorus and of some Phosphorus Compounds. Zeitschr. f. physikal. Chemie 62 (1908), 179.

upon the charged electroscope is especially strong so that there can be no doubt that this trioxide is the exciter of the electrical conductivity of phosphorus air.

Detection of Phosphorous Acid in the Cadaver

The reduction of phosphorous acid to phosphine by zinc and dilute sulphuric acid takes place very slowly. Hilger and Nattermann (loc. cit.) state that even a few milligrams require the action of nascent hydrogen for 10–14 days. Moreover careful manipulation is necessary because silver phosphide is quite unstable. Water decomposes this substance into metallic silver and phosphorous acid and the nitric acid present oxidizes the latter to phosphoric acid. Therefore in a toxicological examination, if special attention must be given to phosphorous acid, Hilger and Nattermann recommend examining the silver precipitate (presumably Ag_3P) after 2 days, or at most 3, for phosphorus by the Dusart-Blondlot method and the filtrate for phosphoric acid (see p. 17).

Hypophosphites.—Various hypophosphites, such as calcium hypophosphite, $Ca(H_2PO_2)_2$, are often used medicinally, for example, in the treatment of children. With nascent hydrogen these compounds yield phosphine and consequently in the absence of phosphorus they may lead to the conclusion that it is present. Hypophosphites according to Th. Panzer[1] are so quickly eliminated that 24 hours after their administration they can no longer be detected in the organism. Consequently a positive result with the Dusart-Blondlot reaction only indicates phosphorus poisoning when it can be shown that the individual did not take hypophosphites internally on the day before his decease. Calcium hypophosphite can be detected in the urine 15 minutes after it has been taken. Such urine imparts a distinct green coloration to the flame when examined by the Dusart-Blondlot method. Apparently this salt passes through the organism without being in any way retained (Panzer).

Examination for Other Volatile Poisons except Phosphorus

When phosphorescence during distillation has been distinctly observed in the Mitschlerlich apparatus, either this operation may be continued, or it may be stopped and distillation resumed with the Liebig condenser in its customary position (Fig. 9). This simpler method of distilling should always be used, when Scherer's preliminary test for phosphorus is negative, or when there is no occasion to test for phosphorus.

Since the several poisons that may appear here are not equally volatile with steam, it is best to collect the distillate in two or three fractions. The first fraction will contain most of the easily volatile substances, such as hydrocyanic acid, chloroform, alcohol, methyl alcohol, iodoform and nitrobenzene. The other fractions may

[1] Th. Panzer: Behavior of Calcium Hypophosphite in the Animal Body. Zeitschr. f. Unters. d. Nahrgs.- und Genussm. 5 (1902), 11.

contain considerable quantities of such substances as carbolic acid, aniline, chloral hydrate, formaldehyde and carbon disulphide that volatilize less easily with steam and so distil over more slowly. This must not be understood to mean that the first part of the distillate will be free from substances that volatilize with difficulty, and the latter part free from those that volatilize easily. In the main such will be the separation, but either part of the distillate may contain traces of substances that will appear in larger quantity in the other part.

The correct procedure is to distil until 5–10 cc. of liquid have been collected. Divide this distillate into several portions and test

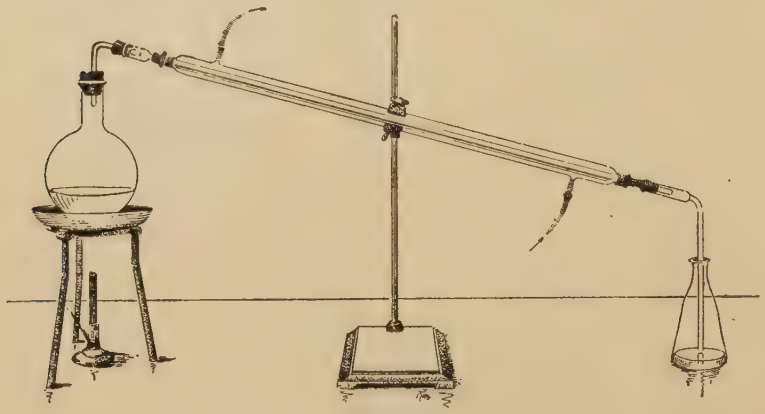


FIG. 9.—Simple distillation apparatus with Liebig condenser.

for hydrocyanic acid, chloroform and alcohol, and, if necessary, also for iodoform and nitrobenzene. Then distil off 10–20 cc. more and use this distillate to test for carbolic acid, cresols, aniline, chloral hydrate, formaldehyde, carbon disulphide and other volatile substances.

Several of these volatile substances have a characteristic odor, such as carbolic acid, nitrobenzene, alcohol, chloroform, iodoform and hydrocyanic acid, which makes it possible to recognize them with great certainty in the original material and especially in the distillate, provided more than traces are present. First, test the distillate for each individual substance by its most characteristic reaction. Test for hydrocyanic acid by the Prussian blue or sulphocyanate reaction; for volatile phenols and aniline with Millon's reagent; for

alcohol, acetone and acetic aldehyde with iodine and potassium hydroxide solution; for chloroform, iodoform and also chloral hydrate with aniline and potassium hydroxide solution, that is, by the phenyl-isocyanide reaction; and finally for carbon disulphide with lead acetate and potassium hydroxide solution. If there is reason to believe that a volatile poison is present, confirm the result by making other characteristic tests. It is seldom necessary to examine the distillate for all the members of the group.

HYDROCYANIC ACID

Hydrocyanic acid, HCN, and various cyanides, such as potassium cyanide, KCN, and sodium cyanide, NaCN, belong to those poisons frequently used in poisonings and suicides because their swift and sure action is generally known. Inasmuch as cyanides of the alkalies are frequently used in electro-metallurgical processes, metallurgy and photography, they are quite easily accessible. Improper medicinal use of bitter almond water and cherry laurel water has also led to poisonings. Both of these preparations contain 0.1 per cent. of hydrocyanic acid, present in part as the cyanohydrin of benzaldehyde. The ferro- and ferri-cyanides, such as yellow and red prussiate of potash, as well as Prussian blue, are practically non-toxic, for with the dilute acid of the gastric juice at body-temperature they give at most only traces of hydrocyanic acid. Usually genuine "Kirschwasser"[1] and "Zwetschgenwasser"[2] also contain hydrocyanic acid. By the colorimetric method the author has found 30 milligrams of hydrocyanic acid in 100 cc. of genuine Black Forest "Kirschwasser." Tobacco smoke frequently contains traces of hydrocyanic acid. According to Habermann,[3] it is not present in pipe smoke, whereas the smoke of Austrian cigarettes contains 0.0023–0.0037 per cent. of hydrocyanic acid and that of cigars 0.0012 per cent. Pontag[4] by smoking 100 grams of Russian tobacco obtained 8 milligrams of hydrocyanic acid and Thoms[5] by smoking

[1] Cherry brandy.

[2] Plum brandy.

[3] Habermann: Contributions to the Knowledge of Cigar Smoke. Zeitschr. f. physiol. Chemie. 33 (1901), 55 and 40 (1904), 148.

[4] J. J. Pontag: Investigation of Russian Tobacco Smoke and of Cigarette Smoke. Zeitschr. f. Untersuchung d. Nahrungs- und Genussmittel 6 (1903), 673.

[5] H. Thoms: The Products in Tobacco Smoke. Ber. d. Deutsch. pharm. Ges. 10 (1900), 19.

15 of Wendt's patent cigars, having a total weight of 73 grams, obtained 3.4 milligrams of Prussian blue.

In a chemical examination for hydrocyanic acid and simple cyanides, such as sodium or potassium cyanide, the contents of the stomach and intestines of the cadaver are most important. In addition organs rich in blood, such as liver, brain and heart, the blood itself and sometimes the urine are also taken. Examine such material at once for hydrocyanic acid which may be recognized by its characteristic odor, provided putrefaction has not gone too far. First make the following preliminary test:

Preliminary Test.—A special test (Schönbein-Pagenstecher) for hydrocyanic acid should precede distillation. Acidify a portion of the original material in a small flask with tartaric acid solution. Then suspend in the flask (see Fig. 1, page 2) a strip of "guaiac-copper" paper[1] without letting it touch the liquid. Gently warm the contents of the flask upon the water-bath. Neither hydrocyanic acid nor potassium cyanide is present, unless the paper is turned blue or bluish green. But the only conclusion to be drawn from a positive test is that hydrocyanic acid, or an easily decomposable cyanide, may be present. Further conclusions should not be drawn from a positive result, since other substances such as ammonia, volatile ammonium compounds, hydrochloric acid, and especially oxidizing agents such as ozone, hydrogen dioxide, nitric acid, and chlorine will turn the paper blue. Consequently, though very delicate, this test cannot be accepted as conclusive proof of the presence of hydrocyanic acid.

The ozonizing action of hydrocyanic acid in conjunction with copper sulphate may be explained by the following equations:

$$(\alpha) \qquad 6CuSO_4 + 12HCN = 6Cu(CN)_2 + 6H_2SO_4.$$
$$(\beta) \quad 3Cu(CN)_2 + 3CuSO_4 + 3H_2O = 6Cu(CN) + 3H_2SO_4 + O_3.$$

These two equations taken together are as follows:

$$(\gamma) \quad 9CuSO_4 + 12HCN + 3H_2O = 3Cu(CN)_2 + 6Cu(CN) + 9H_2SO_4 + O_3.$$

To apply the very delicate guaiac test in detecting hydrocyanic acid in distillates, add first to the distillate a drop of freshly prepared, dilute alcoholic guaiac tincture and then a drop of copper sulphate solution. If the distillate

[1] Prepare "guaiac-copper" paper by saturating strips of filter paper with freshly prepared, 10 per cent. alcoholic tincture of resin of guaiac. Dry these strips in air and moisten before using with very dilute aqueous copper sulphate solution (1:1000).

contains hydrocyanic acid, the mixture will turn deep blue, the coloration reaching a maximum at once. After some time the intensity of color of the blue solution considerably diminishes.

The actual chemical examination for hydrocyanic acid is made by adding tartaric acid solution to the finely divided material and distilling as described (see p. 26). This acid volatilizes easily with steam and most of it will appear in the first part of the distillate. Therefore use the first 10–15 cc. of distillate for the tests. Note cautiously the odor of the distillate, which is characteristic, and then proceed as follows:

1. **Prussian Blue Test.**—Add to the solution (distillate) a little potassium hydroxide solution; then 1–2 drops of freshly prepared ferrous sulphate solution and 1 drop of ferric chloride solution. Shake well and warm gently. Finally acidify with dilute hydrochloric acid. If much hydrocyanic acid is present, a precipitate of Prussian blue will appear at once. But if the quantity is small, the colloidal solution will have merely a blue, blue-green or green-blue color. After a long time (10–12 hours) a flocculent precipitate of Prussian blue will settle to the bottom of the test-tube. The limit of delicacy of this test is 1: 50,000.[1]

Explanation.—Hydrocyanic acid and potassium hydroxide form potassium cyanide which with ferrous sulphate produces ferrous cyanide (α). The latter combines with more potassium cyanide, forming potassium ferrocyanide (β) which with ferric chloride precipitates Prussian blue (γ), the ferric salt of hydroferrocyanic acid, $H_4Fe(CN)_6$:

$$(\alpha) \qquad FeSO_4 + 2KCN = Fe(CN)_2 + K_2SO_4,$$
$$(\beta) \qquad Fe(CN)_2 + 4KCN = K_4Fe(CN)_6,$$
$$(\gamma) \quad 3K_4Fe(CN)_6 + 4FeCl_3 = Fe_4(Fe(CN)_6)_3 + 12KCl.$$

Prussian blue will not appear in presence of alkalies, since they decompose it as follows:

$$Fe_4(Fe(CN)_6)_3 + 12KOH = 3K_4Fe(CN)_6 + 4Fe(OH)_3.$$

Consequently test the final mixture with blue litmus paper, to make sure that it is acid.

Notes.—According to Vorländer,[2] too much hydrochloric acid retards the formation of Prussian blue. In concentrated hydrochloric acid Prussian blue is

[1] A. Link and R. Möckel: Detection of Hydrocyanic Acid. Zeitschr. f. analyt. Chem. 17 (1878), 455.

[2] D. Vorländer: The Prussian Blue Reaction. Ber. d. Deutsch. chem. Ges. 46 (1913), 181.

even soluble with yellow color. If, however, the concentration of the acid is kept below 0.5 normal, its influence upon the formation of Prussian blue may be disregarded. The double decomposition of ferric salts with potassium ferrocyanide in aqueous solution is a time reaction which is retarded or prevented by acids and salts. Therefore the Prussian blue formation does not depend upon a simple instantaneous ionic reaction (Vorländer).

2. Sulphocyanate Test.—Add to a portion of the distillate a few drops of potassium hydroxide solution and then a little yellow ammonium sulphide solution. Evaporate this mixture to dryness in a porcelain dish upon the water-bath. Dissolve the residue in a little water and acidify with dilute hydrochloric acid. Warm and filter through a double paper to remove sulphur. Usually it is necessary to pour the filtrate back upon the filter several times. Add to the clear filtrate 2–3 drops of dilute ferric chloride solution. If the distillate contained hydrocyanic acid, a blood-red color will appear, or a reddish color if only traces of hydrocyanic acid are present. This is due to ferric sulphocyanate. The limit of delicacy of this test is 1: 4,000,000.

Explanation.—Hydrocyanic acid and potassium hydroxide form potassium cyanide which takes sulphur from yellow ammonium sulphide and becomes potassium sulphocyanate (α). The latter in acid solution with ferric chloride forms ferric sulphocyanate (β):

$$(\alpha)\ KCN + (H_4N)_2S_x = KSCN + (H_4N)_2S_{x-1},$$
$$(\beta)\ 3KSCN + FeCl_3 = Fe(SCN)_3 + 3KCl.$$

3. Vortmann's[1] Nitroprusside Test.—Add to a portion of the distillate a few drops of potassium nitrite solution; then 2–4 drops of ferric chloride solution and enough dilute sulphuric acid to give a bright yellow instead of the original yellow-brown color. Heat to boiling, add sufficient ammonium hydroxide solution to remove excess of iron and filter. Add to the filtrate 2 drops of very dilute ammonium sulphide solution. If the solution contained hydrocyanic acid, a violet color will appear and pass through blue, green and yellow. The limit of delicacy of this test is 1: 312,000.

Notes.—This test is the reverse of the nitroprusside test for hydrogen sulphide and is due to conversion of hydrocyanic acid to potassium nitroprusside, $K_2Fe(NO)(CN)_5$, which causes the color changes when ammonium sulphide is added. Very small quantities of hydrocyanic acid give a bluish green to greenish yellow color.

[1] G. Vortmann: A New Reaction for the Detection of Small Quantities of Hydrocyanic Acid. Monatsh. f. Chem. 7 (1886), 416.

4. Silver Nitrate Test.—Acidify a portion of distillate with dilute nitric acid and add silver nitrate solution in excess. If a white, curdy precipitate of silver cyanide (AgCN), easily soluble in ammonia, is formed, it is quite probable that the distillate contains hydrocyanic acid. The limit of delicacy of this test is 1:200,000.

The possibility of mistaking hydrochloric acid for hydrocyanic acid is excluded, if a very dilute solution was distilled, for free hydrochloric acid does not distil over under these conditions. To eliminate every trace of free hydrochloric acid that may be present, redistil the distillate once over borax. This will retain hydrochloric but not hydrocyanic acid. It is advisable to collect upon a filter, wash and dry the precipitate produced by silver nitrate solution. Silver cyanide heated in a bulb-tube forms metallic silver and cyanogen gas. With great caution note the characteristic odor of the gas, or better ignite it at the end of the bulb-tube and observe whether it burns with a purple flame. The decomposition is expressed by the following equation:

$$2AgCN = 2Ag + (CN)_2.$$

When a distillate contains hydrogen sulphide, which is the case when parts of a cadaver that has begun to putrefy are distilled, shake the precipitate produced by silver nitrate with ammonia, filter off the insoluble silver sulphide, and acidify the filtrate with dilute nitric acid. If hydrocyanic acid is present, pure white silver cyanide will now be precipitated.

5. Picric Acid Test.—Render a portion of distillate faintly alkaline with potassium hydroxide solution and warm gently with a few drops of picric acid solution. If hydrocyanic acid is present, the color of the solution will become red. This is due to formation of potasssium isopurpurate.

Waller[1] mixed approximately equal volumes of distillate and an aqueous solution containing 0.05 per cent. of picric acid and 0.5 per cent. of sodium carbonate and allowed the mixture to stand for 1 hour in an incubator at 40°. In this manner he was able to prove that in poisoning by hydrocyanic acid the poison is located mainly in the heart and brain.

Notes.—This picric acid test is not specific for hydrocyanic acid, for other reducing substances such as aldehydes, acetone, hydrogen sulphide and sulphurous acid will also produce a red color with alkaline picric acid solution. For this

[1] A. D. Waller: A New Method for the Quantitative Estimation of Hydrocyanic Acid in Plant and Animal Tissues. Proc. Roy. Soc. London 82 (1910), 574.

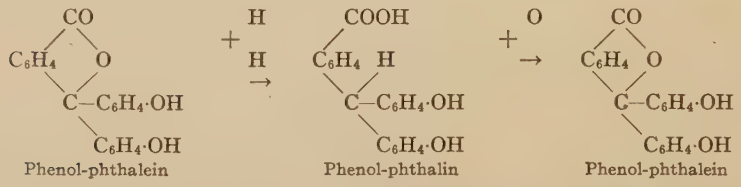
reason a negative result with this test is of greater significance than one that is positive.

6. Phenol-phthalin Test.—Add a few drops of phenol-phthalin dissolved in dilute sodium hydroxide solution and then a little copper sulphate solution (1:2000) to a portion of the distillate. Even in a dilution of 1:500,000 hydrocyanic acid will produce a beautiful red color due to oxidation of phenol-phthalin to phenol-phthalein. Nitric acid, hydrogen dioxide and ferric chloride do not give this test.

Paper first moistened with alkaline phenol-phthalin solution and then with very dilute copper sulphate solution may be used. This paper turns red in air containing hydrocyanic acid.

A phenol-phthalin solution suitable for the detection of hydrocyanic acid may be prepared by boiling a solution of phenol-phthalein in dilute potassium or sodium hydroxide solution with zinc dust until the color is discharged and quickly filtering the cold liquid. This colorless, or nearly colorless filtrate, may be used for the detection of hydrocyanic acid.

Explanation.—Phenol-phthalein is reduced by nascent hydrogen to phenol-phthalin. The latter by ozone and also by hydrocyanic acid in conjunction with copper sulphate is again oxidized to phenol-phthalein which in alkaline solution produces the red color:



To make the recognition of hydrocyanic acid in distillates from parts of the cadaver, or in any other material, more certain and not open to question, the author always uses the Prussian blue and sulphocyanate tests. The other tests described above are not specific for hydrocyanic acid and consequently in the forensic detection of this acid they are of subordinate significance.

Distillation of Hydrocyanic Acid in a Current of Carbon Dioxide and Quantitative Estimation

Bischoff[1] upon the basis of his results from many experiments recommends the following procedure as best adapted for the quanti-

[1] C. Bischoff: Distribution of Poisons in the Human Organism in Cases of Poisoning. Ber. d. Deutsch. chem. Ges. 16(1883), 1337.

tative separation of hydrocyanic acid. The material acidified with tartaric acid and advantageously mixed with alcohol is distilled in a current of carbon dioxide or air. At the same time the distillate is caught in a concentrated solution of silver nitrate. If the distillate is not passed into such a silver solution, loss of hydrocyanic acid occurs even with the best cooling. Distillation in a current of carbon dioxide is carried on in the apparatus shown below (Fig. 10).

Introduce the comminuted material with a small quantity of water into the distilling flask (a) provided with a two-hole stopper and two right-angle tubes, one of which extends almost to the bottom of the flask and the other is in connection with a Liebig condenser (b). A carbon dioxide generator (c) is connected with a wash-bottle. The receiver is an Erlenmeyer flask, containing a few cc. of

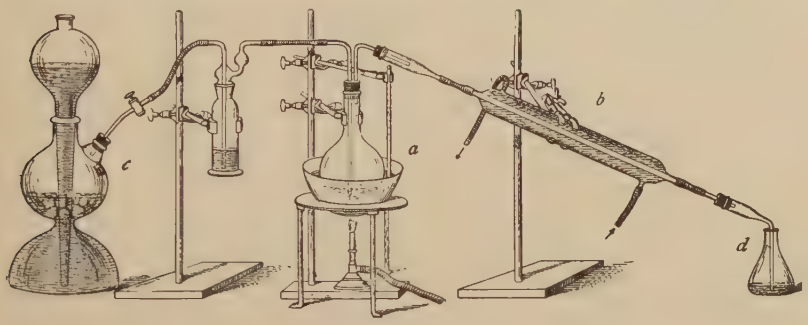


FIG. 10.—Apparatus for distillation of hydrocyanic acid in a current of carbon dioxide.

water into which the end of the adapter (d) dips. It is advisable to set the receiver in ice-water.

In quantitative estimations take a weighed quantity of the well-mixed and comminuted material and instead of water put into the Erlenmeyer flask a few cc. of saturated silver nitrate solution, allowing the end of the adapter to dip below the surface. When the apparatus is ready, acidify the contents of the flask with tartaric acid, start the carbon dioxide, and gently warm the flask in a water-bath or oil-bath. A quantitative estimation takes 2–3 hours and at the end of that time all hydrocyanic acid will be in the receiver. Collect the precipitate of silver cyanide upon a filter, wash it with water until free from silver, dry in a weighed porcelain crucible and ignite. From the weight of metallic silver obtained calculate the amount of hydrocyanic acid by multiplying by 0.2505 (Ag:HCN = 107.8:27 = 0.2505).

If an oil-bath is used in a qualitative test for hydrocyanic acid, one and the same portion of material, after the first fraction obtained by gentle warming is tested for hydrocyanic acid, may be used for the detection of less volatile substances such as phenols, chloral hydrate, formaldehyde, aniline and carbon disulphide, by bringing the contents of the flask to an active boil.

Detection of Hydrocyanic Acid in Presence of Potassium Ferrocyanide

When material contains non-toxic potassium ferrocyanide, hydrocyanic acid will appear in the distillate from a solution acidified with tartaric acid. In an experiment where 1 per cent. potassium ferrocyanide solution was distilled with 0.03 gram of tartaric acid, the distillate contained considerable hydrocyanic acid. Carbon dioxide passed into hot, aqueous potassium ferrocyanide solution will liberate hydrocyanic acid even at water-bath temperature (75°). To test for potassium ferrocyanide beforehand, shake some of the original material with water and filter. Test the filtrate with ferric chloride solution and dilute hydrochloric acid. If there is a precipitate of Prussian blue, potassium ferrocyanide is present. To detect free hydrocyanic acid, potassium or sodium cyanide, but not hydrocyanic acid from mercuric cyanide, add to the material acid sodium carbonate in not too small quantity and distil. Even long distillation over a free flame by this method will liberate hydrocyanic acid only from simple cyanides and not from potassium ferrocyanide! (W. Autenrieth[1].)

Detection of Mercuric Cyanide

When an aqueous solution of mercuric cyanide, which is exceedingly poisonous, is distilled with tartaric acid, the distillate will contain hydrocyanic acid only when a large quantity of mercuric cyanide is present. Distillation of 100 cc. of 1 per cent. aqueous mercuric cyanide solution yields a distillate that gives the Prussian blue test distinctly. But if the quantity of mercuric cyanide is less and the solution very dilute (for example, 100 cc. of 0.01 per cent. solution), there will not be a trace of hydrocyanic acid in the distillate, even though the solution is strongly acidified with tartaric acid. If, however, a few cc. of freshly prepared hydrogen sulphide water are added and distillation is resumed, mercuric cyanide will be completely decomposed and the distillate will contain hydrocyanic acid.

Detection of Mercuric Cyanide in Presence of Potassium Ferrocyanide

The method of detecting hydrocyanic acid from simple cyanides, in presence of potassium ferrocyanide, is not applicable to mercuric cyanide. Long distillation, even from saturated acid sodium carbonate solution, gives no trace of hydrocyanic acid. But distillation in presence of not too little acid sodium carbonate, after addition of a few cc. of freshly prepared, saturated hydrogen sulphide solution, liberates hydrocyanic acid from mercuric cyanide but not from potassium

[1] W. Autenrieth: Contribution to the Knowledge of Yellow Prussiate of Potash and the Detection of Hydrocyanic Acid in Presence of Ferrocyanides. Arch. d. Pharmazie. 231 (1893), 99.

ferrocyanide. It is possible to detect CN'-ion by this method, when very little mercuric cyanide is mixed with considerable potassium ferrocyanide. For example, 0.01 gram of mercuric cyanide in 100 cc. of 10 per cent. potassium ferrocyanide solution can be detected. If potassium ferrocyanide is distilled direct with hydrogen sulphide without addition of acid sodium carbonate, the distillate will contain considerable hydrocyanic acid.

Formation of Hydrocyanic Acid from Normal Organic Substances of Animal Organism at Higher Temperature and in Oxidation.

Sulphocyanic acid, HSCN, occurring normally in the human body, is said to undergo decomposition into hydrocyanic acid and persulphocyanic acid, but only in concentrated aqueous solution in absence of mineral acids, or in presence of small quantities of other acids. In presence of potassium sulphocyanate Ganassini[1] has subjected organic substances to distillation, after adding tartaric or sulphuric acid, and been unable to detect hydrocyanic acid in the distillate. In presence of much potassium sulphocyanate, however, the distillate contained traces of free sulphocyanic acid. During distillation this acid as a rule is decomposed in the following manner:

$$HSCN + 2H_2O = CO_2 + NH_3 + H_2S.$$

Consequently in toxicological investigations errors can not arise in this manner on account of the presence of sulphocyanic acid.

Ganassini, however, maintains that he has positively detected hydrocyanic acid in distillates from organs of healthy animals and that this acid was not present beforehand. Consequently this hydrocyanic acid must have come into existence during distillation! Ganassini assumes that these traces of hydrocyanic acid arise as a result of decomposition of protein substances and xanthine bases of these organs taking place at higher temperatures. Over-heating of these substances is probably brought about when through coagulation they become firmly attached to the walls of the distilling flask, dried and finally brought to the temperature of the distilling flask. Haematin in fact at 200° underwent profound decomposition, yielding hydrocyanic acid together with other substances. To eliminate this source of error, distillation should not be conducted over a free flame but in an oil-bath, the temperature of which does not exceed 120°. In such distillations the author has for years used a bath of paraffine oil. He has never been able, however, in distilling parts of organs and blood over a free flame upon an asbestos wire-gauze to establish any formation of hydrocyanic acid, provided the material did not contain this acid at the start.

Plimmer[2] has made the observation, which deserves mention but is of no practical significance in forensic chemistry, that hydrocyanic acid is formed from protein substances as a result of oxidation. Upon oxidation with a mixture of equal volumes of water, concentrated nitric and sulphuric acid, casein upon the average gave 0.74, haemoglobin 0.56, fibrin 0.66, Witte's peptone 0.53, egg albumin 0.60 and gelatin 0.2 per cent. of hydrocyanic acid. Casein gave the

[1] D. Ganassini: Bull. Chim. Farm. 44,508 and 519.

[2] R. H. Aders Plimmer: Formation of Hydrocyanic Acid in the Oxidation of Protein Substances. Journal of Physiology 31 (1904), 65.

same quantity of hydrocyanic acid when oxidized with potassium dichromate and sulphuric acid but only small quantities when oxidized in sulphuric acid solution with potassium permanganate. If protein substances before oxidation are first hydrolyzed with dilute sulphuric acid, they also give the same quantities of hydrocyanic acid mentioned above. The amino-acids, glycocoll, alanine, α-pyrrolidine-carboxylic acid, aspartic acid, as well as guanidine and succinimide, oxidized with nitric-sulphuric acid gave traces of hydrocyanic acid, at most 0.2 per cent., and tyrosine alone 0.79 per cent.

Relative Stability of Hydrocyanic Acid in Cadaveric Putrefaction

According to L. Lewin the length of time during which hydrocyanic acid can be detected in the animal organism depends mainly upon the degree of putrefaction and the quantity of poison that has been taken. As a matter of fact when putrefaction has set in quite actively, the poison very quickly disappears from the cadaver. In view of these statements a case described by W. Autenrieth[1] is of interest. Weighable quantities of hydrocyanic acid were obtained from the internal organs taken from the exhumed body of a five year old child who had been in the ground for 45 days. These particular internal organs (liver, spleen, kidneys and heart) after this long period were surprisingly well preserved and without any of the strong odor characteristic of decomposition. The putrefactive experiments made by Autenrieth (loc. cit.) with parts of organs and blood, to which bitter almond water or potassium cyanide had been added, are also in agreement with this statement. The results of these experiments showed that hydrocyanic acid in contact with parts of animal organs undergoing decomposition is partially destroyed. This decomposition, however, requires a very long time to come to completion, for even after 60 days there was still present in the mixtures that were in a high state of putrefaction more than 40 per cent. of the hydrocyanic acid originally added. The concentration of the hydrocyanic acid added to these putrefying mixtures apparently has an influence upon the quantity of the acid remaining unchanged, for hydrocyanic acid has a strong toxic action upon ferments and bacteria. In accordance with this view, processes of putrefaction begin more slowly in a 1:10,000 concentration of hydrocyanic acid and proceed less actively than in the lower concentration of 1:20,000. Addition of grape sugar to the putrefying mixtures had no considerable influence upon the quantity of hydrocyanic acid destroyed or combined, not even when the quantity of sugar added was ten times greater than that normally present in human blood.

Cram[2] has reported a fatal case of poisoning caused by whiskey containing potassium cyanide. Five days after death 0.053 gram of potassium cyanide was found in the stomach-contents, this quantity being calculated for the total contents. There was still 0.039 gram of cyanide present 25 days after death and not until 76 days had elapsed could no trace of the poison be detected. In the viscera,

[1] W. Autenrieth: Detection of Hydrocyanic Acid in an Exhumed Body and the Relative Stability of Hydrocyanic Acid during Putrefaction. Ber. d. Deutsch. Pharm. Ges. 20 (1910), 432.

[2] M. P. Cram: A Case of Potassium Cyanide Poisoning. Journ. Amer. Chem. Soc. 36 (1914), 606.

on the other hand, a short time after death not a trace of hydrocyanic acid could be detected.

According to Jollyman[1] also, the stability of potassium cyanide in the stomach after death is far greater than is generally supposed. Even after six months the stomach contents of a negro, who had died from potassium cyanide poisoning, gave distinct tests for hydrocyanic acid. In another experiment Jollyman obtained 0.5 gram of potassium cyanide from a hog weighing 70 kilograms; since the animal did not die, it was shot 1.5 hours after the experiment began. Seven weeks later hydrocyanic acid could still be distinctly recognized in the stomach of the same hog.

Hydrocyanic Acid Poisoning; Symptoms and Lethal Dose

In the case of man, poisoning from hydrocyanic acid, potassium or sodium cyanide, may have a fatal termination very quickly, almost immediately after the poison has been taken, or death may not ensue until several hours have elapsed. Consequently the symptoms exhibited in a poisoning of this kind may be extremely varied. In the first type of poisoning, that is, when a considerable quantity of hydrocyanic acid or of a poisonous metallic cyanide has been taken in one dose, the person poisoned drops unconscious at once after taking the poison and the pupils are greatly enlarged. He takes at most but a few breaths and dies almost immediately. When the poison is taken internally in doses that are not so large but still sufficient to cause death, the symptoms that appear are: dizziness, impairment of the mental faculties, headache, dimness of the field of vision, rush of blood to the head, uneasiness, oppression, pains in the chest and palpitation of the heart. At a more advanced stage of poisoning, there appear impairment of respiration, total unconsciousness and violent convulsions. Finally respiration ceases entirely, whereas the heart continues to beat to the very end (R. Kobert).

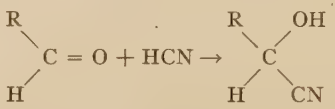
The lethal dose of anhydrous hydrocyanic acid is placed at 0.06 gram for an adult and that of pure potassium cyanide at 0.15 gram. The lethal dose of commercial potassium cyanide, which frequently contains quite considerable quantities of potassium cyanate and carbonate, is placed at 0.25–0.35 gram and that of bitter almond water and cherry laurel water at about 60 grams. The fact that 0.24 gram of commercial potassium cyanide taken internally and 0.3 gram as an enema have caused the death of adults is in accord with the generally accepted view.

Absorption and Toxic Action of Hydrocyanic Acid

In whatever way applied, hydrocyanic acid is easily absorbed, even from the skin. So rapid is the absorption of this poison that there is evidence of an intoxication after a few seconds, or a few minutes at most. This is especially true when a fatal dose of anhydrous hydrocyanic acid is given internally to a man or animal. Part of the poison thus absorbed passes from the body unchanged by way of the lungs. Another part, usually much less, is eliminated by the kidneys and passes into the urine. Sweat also is said to contain hydrocyanic

[1] W. H. Jollyman: The Stability of Potassium Cyanide in the Stomach after Death. Chemiker-Zeitung Jahrgg. 29, 350.

acid. Otherwise, views with regard to the fate of hydrocyanic acid in the animal organism vary widely. Thus it is assumed that hydrocyanic acid takes part in the formation of cyanohydrins with the dextrose of the blood and with other carbohydrates of the animal organism:



and that for this reason it can no longer be detected in the cadaver by means of the usual chemical reactions. The assumption also that hydrocyanic acid combines direct with the proteins of the body or with the loosely bound sulphur of proteins to form derivatives of sulphocyanic acid:

$$HCN + S \rightarrow HSCN$$

is suggested as possible. Finally, hydrocyanic acid may also be oxidized, or hydrolyzed through the agency of enzymes and converted into ammonium formate:

$$HCN + 2H_2O \rightarrow HCOONH_4.$$

All of these hypotheses, regarded from the purely chemical standpoint, are more or less plausible. In addition, it will be shown below that in fatal poisoning a not inconsiderable part of the hydrocyanic acid can still be found as such in the human body and even in the exhumed cadaver.

Hydrocyanic acid acts as a poison for animal and plant protoplasm. For example, it makes impossible the germination of seeds of various plants, although not permanently, but suspends it as long as the poison is present. That is, hydrocyanic acid paralyzes plant enzymes that take part in the germination of seeds. It does not paralyze the assimilating but the dissimilating enzymes. The effect of hydrocyanic acid upon human metabolism is quite similar. It interferes particularly with the action of that enzyme which causes transfer of oxygen from blood-corpuscles and thereby gives rise to oxidative processes (oxidation ferment) of the animal body. Geppert has shown by very careful experiments in metabolism that warm-blooded animals under the influence of hydrocyanic acid take up less than the normal amount of oxygen and consequently give off less carbon dioxide, even though relatively large quantities of oxygen are administered artificially. Consequently the toxic action of hydrocyanic acid depends upon an internal asphyxiation of the organs in presence of an excess of oxygen. The oxidative processes of the blood are checked and so little oxygen is used that the venous blood becomes arterial, that is, contains a large quantity of oxyhaemoglobin. As a result the color of the venous blood is bright red. The occurrence of lactic acid in the blood and a decrease in its alkalinity are also attributable to the severely disturbed oxidative processes of animal metabolism brought about under the influence of hydrocyanic acid. The processes of normal metabolism cause complete oxidation of lactic acid to carbon dioxide and water. Consequently the appearance of lactic acid in the blood of warm-blooded animals is at most transitory and it is not found in the urine at all. As a result of very deficient oxidation in the animal organism during hydrocyanic acid poisoning, not only lactic acid but not infrequently dextrose appears in the urine.

Autopsy in Hydrocyanic Acid Poisoning

The blood and all the body cavities have a distinct odor like that of hydrocyanic acid. If some time has elapsed since death, the odor of hydrocyanic acid in the blood and in the abdominal cavity may not be noticeable. In such cases, however, it can frequently still be distinctly recognized in the cranial cavity, at least as soon as it is opened. The blood as a result of the change of venous to arterial blood has a striking bright red color. Moreover there is only a little coagulum in hydrocyanic acid blood and that is loose. For this reason, it usually remains fluid and mobile for a considerable time. This behavior of hydrocyanic acid blood is to be attributed to the fact that this acid in the capacity of an enzyme poison has more or less checked the enzymatic process of fibrin formation. In hydrocyanic acid poisoning the autopsy shows further that the blood-vessels of the brain and the right heart are usually entirely full. The lungs, liver and kidneys may also show that the blood-vessels are very full of blood. The bronchial tubes may be reddened and filled with reddish froth. In poisonings from free hydrocyanic acid the gastric mucosa, except for some slight extravasation of blood, may appear unchanged. In the case of potassium and sodium cyanide, the action of caustic alkalies is noticeable, for these salts are considerably hydrolyzed:

$$KCN + HOH = HCN + KOH$$

The color of the death-spots is more or less characteristic of hydrocyanic acid poisoning. In more than 30 per cent. of the cases of hydrocyanic acid poisoning, they do not have a dark but a striking bright red color that would at once be noticed even by a novice.

PHENOLS

General Behavior and Reactions

All phenols dissolve in aqueous solutions of alkaline hydroxides forming phenolates:

$$C_6H_5.OH + NaOH = C_6H_5.ONa + H_2O.$$

and acids, even carbon dioxide, reprecipitate them from these solutions:

$$C_6H_5.ONa + HCl = C_6H_5.OH + NaCl.$$

This solubility in solutions of alkaline hydroxides is due to the presence of one or more hydroxyl-groups in the benzene ring. Most of the monacid phenols are volatile with steam. For this reason, they can be separated from other materials by distillation with water and they then impart to the distillate their more or less specific odor. Belonging to this class of phenols, are such substances as carbolic acid, the cresols, guaiacol and creosol, thymol, as well as α- and β-naphthol.

On the other hand, phenols containing more than one hydroxyl-group are practically non-volatile with steam and as a rule are without characteristic odor. Substances of more importance medicinally belong to this group, such as catechol, resorcinol, quinol and pyrogallol.

General Phenol Reactions

1. Millon's Test.—This is also known as Plugge's[1] reaction. With a few drops of Millon's reagent, the aqueous solution of a monacid phenol even in the cold, or when warmed if only traces of the phenol are present, takes on a light to dark red color. This test seems to be specific for monacid phenols and their derivatives. Consequently salicylic acid, p-oxy-benzoic acid, tyrosine, salicylic alcohol and salicylic aldehyde also give Millon's test. In the case of the alkyl ethers of phenols this test is not given until later.

This reaction takes place when one or more hydrogen atoms of the benzene ring of the monacid phenols are replaced by hydrocarbon radicals. But such substituents as chlorine, bromine, iodine or carbon-free radicals prevent it. The fact that aniline and other primary, aromatic amines give this test is due to the presence in the reagent of free nitric and nitrous acid. They first form diazonium salts and these warmed with water give phenols:

$$\text{(a)}\quad C_6H_5.N\,H_2.H\,NO_3 + (N\,OOH) = 2H_2O + C_6H_5.N_2.NO_3,$$

$$\text{(b)}\quad C_6H_5.N_2.NO_3 + (HO\;\;H) = C_6H_5.OH + N_2 + HNO_3.$$

Phenols containing more than one hydroxyl-group do not give the typical red color but some of them reduce Millon's reagent with separation of mercury.

2. Ferric Chloride Test.—Dilute ferric chloride solution imparts a characteristic color to the aqueous solution of many phenols and their derivatives. These colors are blue, blue-violet, red-violet, red and green. But aqueous solutions of substances containing no free phenolic hydroxyl-group, such as antipyrine and pyramidone, also give colors with ferric chloride. In the case of many phenols, such as quinol and the two naphthols, ferric chloride has an oxidizing action. Free dilute hydrochloric, or sulphuric acid, discharges the colors produced by ferric chloride in aqueous solutions of phenols.

[1] P. C. Plugge: Mercuric Nitrate containing Nitrous Acid as a Reagent for Aromatic Compounds containing a Hydroxyl-group in the Benzene Ring. Archiv. d. Pharmazie. 228 (1890), 9.

3. Liebermann's[1] Test.—Dissolve a small quantity of a phenol in concentrated sulphuric acid and add a few drops of a solution of 0.6 gram of potassium nitrite in 10 grams of concentrated sulphuric acid (Liebermann's reagent). Various phenols give different colors that change upon addition of glacial acetic acid, or water, and upon saturation with ammonia. Carbolic acid gives a blue solution that becomes red upon addition of glacial acetic acid, or water, and turns blue again upon saturation with ammonia. Liebermann's test is due to the formation of a colored nitroso-derivative.

4. Eykmann's[2] Test.—This is a modification of Liebermann's reaction. Add to a dilute aqueous solution of a phenol a few drops of an alcoholic solution of ethyl nitrite (Spiritus Aetheris Nitrosi) and then concentrated sulphuric acid from a pipette so that it forms a distinct under-layer. A red zone will appear at the contact-surface of the two liquids. Various colors appear upon addition of glacial acetic acid, or water, and excess of ammonia. In a dilution of 1:200,000 carbolic acid still gives a distinct red color that changes to blue upon addition of ammonia.

5. Azo-Dye Formation.—Diazonium salts, such as diazo-benzene chloride, obtained by adding sodium nitrite to a hydrochloric acid solution of aniline hydrochloride, couple with phenols and naphthols forming oxyazo-dyestuffs:

(a) $C_6H_5.NH_2.HCl + HOON = 2H_2O + C_6H_5.N_2.Cl$,

(b) $C_6H_5.N_2.Cl + H.C_6H_4.OH = HCl + C_6H_5.N_2.C_6H_4.OH$.

Dissolve 2–3 drops of aniline in an excess of dilute hydrochloric acid, cool with ice to 0°. First add to this solution a few drops of 0.5 per cent. sodium nitrite solution, then the faintly alkaline phenol solution, and finally an excess of sodium hydroxide solution. Yellow, scarlet or bordeaux-red colors appear, or colored precipitates, consisting of the azo-dyestuff formed. Under the same conditions many aldehydes and ketones also give more or less pronounced colorations.

6. Schotten-Baumann Benzoylation Test.—In comparison with the general phenol color-reactions already mentioned, this test possesses the advantage, not to be undervalued in forensic chemistry, of giving even with very dilute aqueous phenol solutions well-defined

[1] C. Liebermann: Dyestuffs formed from Aromatic Oxy-Compounds and Nitrous Acid. Ber. d. Deutsch. chem. Ges. 7 (1874), 1098.

[2] Eykmann: Zeitschr. f. analyt. Chemie 22 (1883), 576.

substances that usually crystallize well and melt sharply. By a determination of the melting-point the substance can be more definitely characterized. Add to the given aqueous phenol solution about 10 cc. of sodium hydroxide solution (10 per cent.) and also 1–2 cc. of benzoyl chloride. Shake the mixture vigorously until the odor of benzoyl chloride has disappeared. Throughout the entire test the reaction should be alkaline. If it becomes acid, more sodium hydroxide solution must be added. The ester of benzoic acid that is formed usually separates as a white, crumbly mass insoluble in water. Filter it off and wash with cold water until free from alkali. Frequently it can be crystallized from alcohol or acetone by preparing a saturated solution of the ester at ordinary temperature, mixing with about the same volume of water and setting in ice:

$$C_6H_5.COCl + C_6H_5.OH + NaOH = C_6H_5.CO.OC_6H_5 + NaCl + H_2O.$$

Primary and secondary amines, as well as ammonia, which can also be benzoylated, should not be present. Should the liquid under examination contain such compounds, it must be strongly acidified with dilute sulphuric acid and then distilled. The resulting distillate will contain only phenols. Even from a solution containing an excess of sulphuric acid, traces of aniline will pass over into the distillate. Alcohols usually give liquid benzoyl derivatives. The benzoyl derivatives of the more common phenols and naphthols are the following:

Name	Formula	Crystalline Form	Melting-Point
Phenyl Benzoate	$C_6H_5.COOC_6H_5$	Prisms	68–69°
p-Cresyl Benzoate	$C_6H_5.COOC_6H_4.CH_3$	Plates	71.5°
α-Naphthyl Benzoate	$C_6H_5.COOC_{10}H_7$	Plates or Prisms	56°
β-Naphthyl Benzoate	$C_6H_5.COOC_{10}H_7$	Plates or Prisms	108°

These esters can also be recrystallized from an alcohol-ether mixture.

Supplement to Volatile Phenols

(Maltol[1])

Maltol, $C_6H_6O_3$, is formed in the preparation of caramel from malt, possibly from maltose or isomaltose. Ether or chloroform extracts this substance from the condensed vapors given off during caramelization and also from beer-wort. Maltol crystallizes in monoclinic prisms and plates from a cold, saturated solution in 50 per cent. alcohol. Chloroform gives more compact crystals. Maltol dissolves with difficulty in cold water or benzene; more readily in hot water, alcohol, ether or chloroform; and is insoluble in petroleum ether. It dissolves in caustic alkaline solutions but is reprecipitated by carbon dioxide. Maltol sublimes in shining leaflets but is reprecipitated by carbon dioxide.

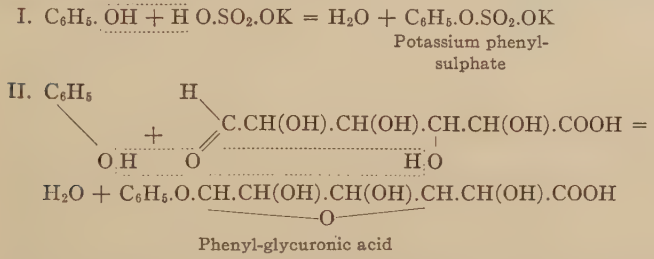
[1] J. Brand: Maltol. Ber. d. Deutsch. chem. Ges. 27 (1894), 806; K. Kiliani and M. Bazlen: Maltol. Ber. d. Deutsch. chem. Ges. 27 (1894), 3115.

It reduces silver solution in the cold and Fehling's solution with heat. Mention should be made of the fact that the aqueous solution of maltol, although this compound is not a phenol, gives an intense violet color with ferric chloride solution. In distinction from the phenols volatile with steam and salicylic acid, a maltol solution does not turn red when warmed with Millon's reagent.

Since maltol gives a mono-benzoyl derivative when shaken with benzoyl chloride and sodium hydroxide solution, its molecule must contain one hydroxyl-group.

Physiological Behavior of Phenols

The phenols also show quite a close resemblance in their behavior in the animal organism in that the latter seeks to get rid of the toxic effects of phenols by quickly eliminating them again after they have been administered and absorbed. Conjugation with sulphuric acid (I) as well as with glycuronic acid (II) is the method followed in getting rid of the poisons. In the form of their alkali salts the conjugated acids thus formed are eliminated in the urine through the kidneys:



As a result of the formation of "conjugated sulphuric acid," which is present in small quantity in every normal human urine, the "sulphate-sulphuric acid," also normally present, in part disappears. Consequently in a poisoning caused by a phenol the conjugated sulphuric acid, known also as ethereal sulphuric acid, is more or less strongly increased. On the other hand, the quantity of sulphate-sulphuric acid in the urine, as compared with that normally eliminated, is diminished. A urine containing conjugated glycuronic acids is laevo-rotatory and reduces Fehling's solution.

In the animal organism monacid phenols are oxidized in part to di-acid phenols. As a consequence "carbolic urine" eliminated, following administration of carbolic acid, contains quinol-sulphuric acid in addition to phenyl-sulphuric and phenyl-glycuronic acid. As a result of severe impairment of the kidneys the sediment of "carbolic urine" frequently contains albumin, blood-pigment, free blood-corpuscles, epithelial and cylindrical cells.

CARBOLIC ACID

Carbolic acid, or benzo-phenol, $C_6H_5.OH$, forms long, colorless, pointed crystals, or a white, radiating crystalline mass having a characteristic odor. Liquid carbolic acid (Acidum Carbolicum Liquefactum) contains 10 per cent. of water and is a clear, colorless

liquid, or has a faint reddish color. On the other hand, carbolic acid dissolves in about 20 parts of water and further is readily soluble in alcohol, ether, and chloroform as well as in solutions of caustic alkalies. Pure anhydrous carbolic acid melts at 40–42° and boils at 178–182°.

Carbolic acid distils relatively easily with steam, but rather prolonged distillation is required to get the last portions over. In most cases it may be recognized by its characteristic odor. When present in larger quantities, colorless or reddish oil-drops usually float about in the milky distillate. These dissolve in potassium or sodium hydroxide solution and the distillate becomes clear. Since small quantities of volatile phenols, especially of p-cresol, are formed during putrefaction of proteins, distillates from parts of cadavers that have passed into a high state of putrefaction almost always contain traces of these two phenols. Such a distillate from putrefied cadaveric material consequently gives Millon's test and usually also the test with bromine water.

The following tests may be used for the purpose of detecting carbolic acid:

1. Millon's Test.—Millon's reagent,[1] heated with a solution containing only a trace of carbolic acid, produces a red color. An aqueous solution containing only 20 milligrams of carbolic acid, diluted 1:100,000, will give a distinct red color. If the phenol solution is not very dilute, the color will appear even in the cold. Though a very delicate test, it is not characteristic of carbolic acid, because very many aromatic substances, especially other monacid phenols and their derivatives resemble carbolic acid in their behavior toward Millon's reagent.[2]

FIG. 11.—Tribromo-phenyl hypobromite crystals from a dilution of 1:20,000.

2. Bromine Water Test.—Excess of bromine water produces a yellowish white, crystalline precipitate, consisting really of tribromo-phenyl

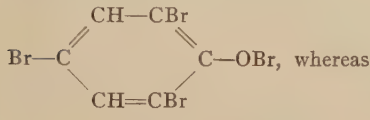
[1] Para-hydroxy-phenyl-acetic acid and hydro-para-cumaric acid are formed in the putrefaction of proteins but are not volatile with steam. Consequently they cannot be present in a distillate that is to be tested for volatile phenols.

[2] Traces of salicylic acid volatilize with steam, at least in such quantity that it can be detected in the distillate with Millon's reagent.

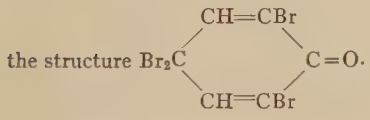
hypobromite, even with very dilute carbolic acid solutions. It is a very delicate test for carbolic acid. Phenol diluted 1:50,000 yields after some time a precipitate made up in part of well-formed crystals (Fig. 11). Pure tribromo-phenyl hypobromite melts at 131–133°, giving off bromine at the same time.

Salicylic alcohol (saligenin), salicylic aldehyde, salicylic acid, and p-hydroxybenzoic acid are also converted quantitatively by an excess of saturated bromine water even in the cold into tribromo-phenyl hypobromite.

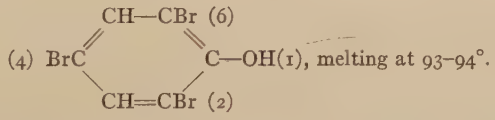
If there is a sufficient excess of bromine water to give the supernatant liquid a brownish red color, the precipitate consists only of tribromo-phenyl hypobromite, $C_6H_2Br_3OBr$. Benedikt[1] regards this compound as a bromo-phenoxy-tribromobenzene having the structure:



whereas Thiele and Eichwede[2] have ascribed to it the structure



This reaction takes place so easily that carbolic acid may even be determined quantitatively as this tetrabromo-derivative (see page 47). It melts at 132–134° with evolution of bromine and crystallizes in lemon-yellow leaflets from alcohol-free chloroform or ligroin. Heated with alcohol, acetone, xylene, or aqueous sulphurous acid, this compound loses bromine and changes at once to 2, 4, 6-tribromo-phenol:



melting at 93–94°.

3. Ferric Chloride Test.—Very dilute ferric chloride solution, added drop by drop, imparts a blue-violet or blue color to aqueous carbolic acid solutions. Addition of dilute hydrochloric, or sulphuric acid, changes this color to yellow. The color also disappears upon addition of alcohol (distinction from salicylic acid). This test is not as delicate as 1 and 2. It is entirely negative in presence of mineral acids. The limit of delicacy is about 1:1000.

[1] R. Benedikt: Bromoxy-Derivatives of Benzene. Annalen d. Chemie 199 (1879), 127.

[2] J. Thiele and H. Eichwede: The Constitution of Tribromo-phenyl Hypobromite. Ber. d. Deutsch. chem. Ges. 33 (1900), 673.

4. **Hypochlorite Test.**—Add a few cc. of ammonium hydroxide solution to a dilute, aqueous carbolic acid solution, and then 2–4 drops of freshly prepared calcium or sodium hypochlorite solution. Gentle warming will produce a blue color. Very dilute carbolic acid solutions after some time give only a green to blue-green color. Flückiger[1] allows bromine vapor to come in contact with the phenol solution that has been mixed with a little ammonium hydroxide solution in a porcelain dish. Ortho- and meta-cresol also give a blue color but that given by para-cresol is a dirty brownish green. This test is also known as Lex's reaction.

5. **Melzer's Benzaldehyde Test.**[2]—Add 2 cc. of concentrated sulphuric acid to 1 cc. of the solution (distillate) to be tested for carbolic acid, then 1–2 drops of benzaldehyde and heat. The liquid that is yellowish brown in the cold takes on a dark red color in presence of phenol. At the same time a red resinous substance appears unless the solution is too dilute. When cold, add 10 cc. of water and enough potassium hydroxide solution to give a distinct alkaline reaction. If carbolic acid is present, a beautiful violet-blue color will appear. To obtain this coloring-matter, acidify the solution, extract with ether and evaporate the solvent. Alkalies added to alcoholic solutions of the coloring-matter produce a blue color that acids discharge. This is a very delicate test. One cc. of 0.5 per cent. carbolic acid solution (= 0.0005 gram of carbolic acid) will still give a distinct blue color. Of the three cresols only o-cresol gives this test.

6. **Formaldehyde Test.**—Pougnet[3] recommends a mixture of 10 cc. of concentrated sulphuric acid, 10 cc. of water, and 20 drops of 40 per cent. formaldehyde solution. This reagent for phenols is used more advantageously as two separate liquids, one of which (No. 1) consists of a mixture of 10 cc. of water and 20 drops of formaldehyde solution and the other (No. 2) of concentrated sulphuric acid. Equal volumes of these two liquids are mixed as required. With phenols, and substances containing at least one phenolic-group, this reagent gives characteristic precipitates or colors. Put about 0.02 gram of the substance in a test-tube and then add 1 cc. of solution No. 1 and 1 cc. of solution No. 2. If the given substance is a liquid, add 1 cc. to 1 cc. of concentrated sulphuric acid and then 2 drops of formaldehyde solution.

[1] Pharmazeutische Chemie 1879, page 287.

[2] H. Melzer: Contributions to Forensic Chemistry. Zeitschr. f. analyt. Chemie 37 (1898), 345.

[3] J. Pougnet: A General Reagent for Phenols. Bull. de Sciences Pharm. 16 (1900), 142; Schweiz. Wochenschr. f. Chem. u. Pharm. 350 (1909).

QUANTITATIVE ESTIMATIONS OF PHENOL

1. Gravimetric Estimation as Tribromo-phenyl Hypobromite

(Autenrieth and Beuttel[1])

This method is based upon the behavior of aqueous phenol solutions toward an excess of saturated bromine water which completely precipitates the phenol as tribromo-phenyl hypobromite, $C_6H_2Br_3OBr$ (see above). Since the latter is practically insoluble in cold bromine water, this method of estimating carbolic acid gives very satisfactory results.

Procedure.—Place the aqueous phenol solution in a large glass-stoppered flask. Add gradually, while shaking, saturated bromine water until the supernatant liquid has a red-brown color and bromine vapor is visible above the solution. Shake frequently and allow to stand for 2–4 hours. Then collect the precipitate in a weighed Gooch crucible and dry in a vacuum desiccator (Fig. 13, see page 96) over sulphuric acid to constant weight. On the basis of the following proportion calculate the weight of phenol corresponding to the weight of the precipitate:

$$\underset{(409.86)}{C_6H_2Br_4O} : \underset{(94.05)}{C_6H_5.OH} = \text{Wt. of Ppt. found} : x$$

Since the ratio $94.05 : 409.86 = 0.2295$, the weight of phenol may be found by multiplying the weight of the precipitate by 0.2295.

2. Beckurts-Koppeschaar[2] Volumetric Method

Dilute sulphuric acid liberates hydrobromic acid from potassium bromide (α) and bromic acid from potassium bromate (β). These two acids react according to (γ) with liberation of bromine:

$$(\alpha) \quad 2KBr + H_2SO_4 = 2HBr + K_2SO_4,$$
$$(\beta) \quad 2KBrO_3 + H_2SO_4 = 2HBrO_3 + K_2SO_4,$$
$$(\gamma) \quad HBrO_3 + 5HBr = 3Br_2 + 3H_2O.$$

Therefore addition of dilute sulphuric acid to a mixture of potassium bromide and bromate solutions liberates bromine that will

[1] W. Autenrieth and Fr. Beuttel: Estimation of Phenol, Salicylic Alcohol, Salicylic Acid, Etc. as Tribromo-phenyl Hypobromite. Arch. d. Pharm. 248 (1910), 112.

[2] H. Beckurts: Quantitative Estimation of Carbolic Acid as Tribromo-phenol. Arch. d. Pharm. 224 (1886), 561–572. W. F. Koppeschaar: Volumetric Estimation of Phenol. Zeitschr. f. analyt. Chemie 15 (1876), 232.

convert phenol into a mixture of tribromo-phenol and tribromo-phenyl hypobromite. The excess of free bromine, as well as the one labile bromine atom of tribromo-phenyl hypobromite, will displace iodine from potassium iodide and finally all the phenol will be present as tribromo-phenol:

$$\underset{\text{Tribromo-phenyl Hypobromite}}{C_6H_2Br_3OBr} + 2KI = \underset{\text{Potassium tribromo-phenolate}}{C_6H_2Br_3OK} + KBr + I_2$$

One molecule of phenol requires 6 atoms of bromine, as shown in the equation:

$$5KBr + KBrO_3 + 6H_2SO_4 + C_6H_5.OH = C_6H_2Br_3OH + 3HBr + 6KHSO_4 + 3H_2O.$$

Titration requires the following standard solutions:

1. 0.01 n-potassium bromide solution, containing $\frac{5KBr \text{ grams}}{100} = \frac{595.6}{100} =$ 5.956 grams of KBr in 1000 cc.

2. 0.01 n-potassium bromate solution, containing $\frac{1KBrO_3}{100} = \frac{167.17}{100} = 1.6717$ grams of $KBrO_3$ in 1000 cc.

3. 0.1 n-sodium thiosulphate solution, containing 0.1 $Na_2S_2O_3.5H_2O$ grams = 24.83 grams in 1000 cc.

4. Potassium iodide solution, containing 125 grams of KI in 1000 cc.

Procedure.—Put about 25 cc. of aqueous phenol solution (distillate) into a flask having a tight glass stopper. Add 50 cc. each of 0.01 n-potassium bromide and 0.01 n-potassium bromate solutions, then 5 cc. of pure concentrated sulphuric acid and shake vigorously for several minutes. The gradually increasing opalescence of the solution becomes more and more marked, as tribromo-phenol and tribromo-phenyl hypobromite are precipitated. The yellow color that soon appears shows excess of bromine. Open the flask after 15 minutes, add 10 cc. of potassium iodide solution, shake and titrate free iodine after a few minutes with 0.1 n-sodium thiosulphate solution.

Calculation.—$\frac{6 \text{ gram-atoms Br}}{100} = \frac{6 \times 79.96}{100} = 4.7976$ grams of bromine are set free from a mixture of 1000 cc. each of 0.01 n-potassium bromide and 0.01 n-potassium bromate solution. A mixture therefore of 50 cc. each of the two solutions will give 0.23988 gram of bromine. This quantity of bromine can convert 0.04704 gram of phenol into tribromo-phenol:

$$6Br : C_6H_5OH$$
$$479.76 \quad 94.05 = 0.23988 : x \quad (x = 0.04704)$$

1 cc. of 0.1 n-sodium thiosulphate solution corresponds to 0.012697 gram of iodine and this quantity of iodine to 0.007996 gram of bromine. But 0.007996 gram of bromine will convert 0.00157 gram of phenol into tribromo-phenol:

$$6Br : C_6H_5OH$$
$$479.76 \quad 94.05 = 0.007996 : x \quad (x = 0.00157)$$

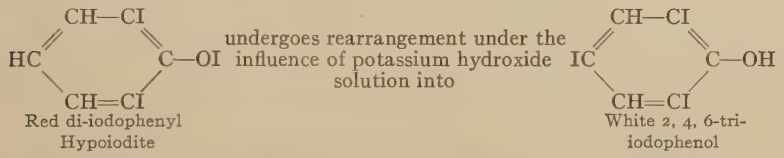
Consequently, for each cc. of 0.1 n-sodium thiosulphate solution used, subtract 0.00157 from 0.04704 gram of phenol. This determines the quantity of carbolic acid in the 25 cc. of distillate taken.

3. Messinger-Vortmann[1] Volumetric Method

Excess of iodine (8 atoms of iodine to 1 molecule of phenol dissolved in 4 molecules of potassium hydroxide), added to a moderately warm alkaline phenol solution, will produce a dark red, non-crystalline precipitate. One molecule of phenol requires 6 atoms of iodine:

1. $C_6H_5OH + 3I_2 = C_6H_2I_3OH + 3HI$,
2. $3HI + 3KOH = 3H_2O + 3KI$.

This red precipitate dissolves in hot potassium hydroxide solution with a red-brown color and appears as white 2, 4, 6-tri-iodophenol, melting at 154–156°, on addition of an excess of dilute sulphuric acid. Messinger and Vortmann regard the red compound as di-iodophenyl hypoiodite, $C_6H_3I_2OI$, which potassium hydroxide converts into the more stable isomeric tri-iodophenol:



Red di-iodophenyl Hypoiodite

White 2, 4, 6-tri-iodophenol

Procedure.[2]—The reaction between alkaline phenol solution and iodine is rather slow in the cold but is hastened at 50–60°.

Place a measured volume of aqueous phenol solution (5–10 cc.) in a small flask and add a measured volume of 0.1 n-potassium hydroxide solution until the mixture is strongly alkaline. Warm gently by dipping the flask in water at 60° and add 10–15 cc. more of 0.1 n-iodine solution than the volume of 0.1 n-potassium hydroxide solution used, or until the excess of iodine produces a strong yellow color. Agitation will cause a deep red precipitate to appear. Cool the solution, acidify with dilute sulphuric acid, and dilute to a defi-

[1] J. Messinger and G. Vortmann: A New Class of Iodated Phenols. Ber. d. Deutsch. chem. Ges. 22 (1889), 2312; and Volumetric Estimation of Phenols. Ibid. 23 (1890), 2753. See also Kossler and Penny, Zeitschr. f. physiolog. Chemie 17 (1892), 117.

[2] Use 0.5–1 per cent. carbolic acid solution for laboratory experiments.

nite volume (250–500 cc.). Filter an aliquot portion (100 cc.) rapidly and determine excess of iodine with 0.1 n-sodium thiosulphate solution.

Calculation.—Each molecule of phenol requires 6 atoms of iodine. Therefore 1 atom of iodine $= \frac{C_6H_5OH}{6} = \frac{94.05}{6} = 15.675$ grams of phenol. 1000 cc. of 0.1 n-iodine solution, containing 0.1 gram-atom of iodine, correspond therefore to 1.5657 grams of phenol.

Note.—This method will not give satisfactory results, unless at least 3 molecules of sodium or potassium hydroxide are taken for 1 molecule of phenol.

Estimation of Phenol in Urine[1]

In determining carbolic acid in urine, the normal occurrence of phenols must not be overlooked. After a mixed diet, the quantity of normal human urine passed in 24 hours will yield approximately 0.03 gram of phenols (phenol and more especially p-cresol).

In certain diseases where there is excessive bacterial decomposition within the organism, in the intestines for example, urine contains more of these phenols and consequently more conjugated sulphuric acids. Even external application of carbolic acid, for instance the use of carbolic acid water as a lotion, is sufficient to increase the quantity of phenyl-sulphuric acid in urine.

Detection of Carbolic Acid in Presence of Aniline

Aniline closely resembles carbolic acid in behavior toward Millon's reagent and bromine water. But the two substances can be easily separated. Add potassium hydroxide solution in large excess and distil. The distillate will contain aniline alone. Or make the solution strongly acid with dilute sulphuric acid and extract with ether which will dissolve only carbolic acid. Evaporate the ether extract at a moderate temperature and examine the residue.

Toxic Action and Elimination of Carbolic Acid

Concentrated carbolic acid coagulates and destroys the constituents of the human body, especially proteins and protoplasmic structures. It has therefore a very strong caustic action. But its action is not merely local, for after absorption it shows an affinity particularly for the central nervous system, brain and spinal cord. The first indications of this in animals are strong stimulation, increased irritability as in the case of strychnine, and paralysis. In man the period of stimulation is very slow in appearing. In chronic poisoning, after repeated small doses of carbolic acid, degeneration of the kidneys and liver is a result of absorption. The human organism absorbs carbolic acid very rapidly. Absorption from the skin, the gastro-intestinal tract, abrasions and the respiratory organs takes place readily. In the human organism the poison is converted by conjugation with acid potassium sulphate into potassium phenyl-sulphuric acid, $(C_6H_5)O.SO_2.OK$. When the quantity of carbolic acid is very large, it is

[1] The methods of estimating volatile phenols of the urine are taken up in Chapter V, page 557 of this book.

also converted into phenyl-glycuronic acid by conjugation with glycuronic acid, HOOC.(CH.OH)$_4$.CHO. Considerable carbolic acid is oxidized within the body to dihydroxy-benzenes, namely catechol, $C_6H_4(OH)_2$(1, 2), and quinol, C_6H_4-$(OH)_2$(1, 4). These enter into synthesis with sulphuric acid and appear in the urine as ethereal salts of sulphuric acid. The dark color of "carbolic urine" is largely due to further oxidation of quinol, whereby colored products (quinone?) are formed. In carbolic acid poisoning, urine often has a pronounced dark color (greenish to black). Urine in other cases is amber-yellow at first, but standing in air gives it a deeper color. When carbolic acid poisoning is suspected, the urine should be examined chemically. "Carbolic urine"[1] differs from normal human urine in being nearly free from "sulphate-sulphuric acid,"[2] the so-called "pre-formed sulphuric acid." Consequently barium chloride solution, in presence of excess of acetic acid, gives only a slight precipitate of barium sulphate or none at all. Filter when there is a precipitate and warm the clear filtrate with a few cc. of concentrated hydrochloric acid. An abundant precipitate of barium sulphate will usually appear. The mineral acid decomposes phenyl-sulphuric acid into phenol and sulphuric acid which is then precipitated. Normal human urine contains considerably more "sulphate-sulphuric acid" (A-sulphuric acid) than "ethereal sulphuric acid" (B-sulphuric acid). The average proportion between the two being: A-SO_4: B-SO_4 = 10: 1. Barium chloride solution, added to normal urine in presence of acetic acid, produces a heavy precipitate of barium sulphate.

Distribution of Carbolic Acid in Human Body after Fatal Poisoning

Bischoff[3] examined organs, removed from a man who had died 15 minutes after taking 15 cc. of liquid carbolic acid, and found the poison distributed as stated in the table below. The organs in this case were perfectly fresh. Only a small portion of the stomach was received.

Weight	Organ	Phenol
242 grams	Contents of stomach and intestine	0.171 gram
112 grams	Blood	0.028 gram
1480 grams	Liver	0.637 gram
322 grams	Kidney	0.201 gram
1445 grams	Brain	0.314 gram

Bischoff distilled with steam until the distillate gave no further precipitate with bromine water. The results show how rapidly carbolic acid is absorbed and how soon it is distributed throughout the body.

Urine gives a distinct test for carbolic acid 15 minutes after the poison has been taken by the mouth, or hypodermically. This shows how rapidly carbolic

[1] The methods of examination of "Carbolic Urine" and the estimation of "Sulphate-Sulphuric Acid" and the conjugated sulphuric acid are taken up in Chapter V, page 559 of this book.

[2] This sulphuric acid, occurring in the urine in the form of sulphates, gives SO_4''-ions.

[3] C. Bischoff: Distribution of Poisons in the Human Organism in Cases of Poisoning. Ber. d. Deutsch. chem. Ges. 16 (1883), 1337.

acid is absorbed. Most of the carbolic acid absorbed is eliminated in 4–5 hours. Schaffer[1] found the quantity of conjugated sulphuric acid in urine to increase in exact proportion to the quantity of carbolic acid taken.

Baumann[2] has published certain facts relating to the quantity of carbolic acid formed during putrefaction of protein substances. Baumann states that he obtained from 100 grams of fresh pancreas and 100 grams of moist fibrin, mixed with 250 cc. of water, after 6 days of putrefaction 0.073–0.078 gram of tribromophenol, corresponding to 0.0208–0.022 gram of phenol. Most of the volatile phenols formed during the putrefaction of proteins consists of p-cresol. Brieger[3] determined that 0.72 gram of phenol was the maximum quantity formed as the result of 6 days putrefaction of a liver weighing 2 kilograms. If putrefaction was allowed to go on longer, the quantity of phenol decreased.

CRESOLS AND LYSOL

The three cresols, $C_6H_4(CH_3)OH$, together with a small quantity of carbolic acid and hydrocarbons are the chief components of crude coal-tar creosote. Since this mixture is only slightly soluble in water, addition of potash soaps, alkali salts of aromatic acids such as sodium cresotinate, $HO.C_6H_3(CH_3)COONa$, renders it soluble. Lysol belongs to preparations of this kind which are frequently used in the treatment of wounds and for purposes of disinfection. As a matter of fact it is identical with the *Liquor Cresoli Saponatus* of the German Pharmacopoeia by which is understood a solution prepared by warming 1 part of crude cresol with 1 part of a potash soap from linseed oil. It is a clear, brown liquid having a cresol-like odor and giving a clear mixture with equal volumes of alcohol, ether and chloroform. When shaken with twice its volume of water, lysol gives a gelatinous mass that dissolves in more water forming a liquid that foams freely. Dilute ferric chloride solution produces a transitory blue-violet color and then a dirty turbidity. Addition of an excess of hydrochloric acid precipitates the cresols and fatty acids as a brown oily liquid.

Detection of Cresols

For the chemical confirmation of suspected poisoning by lysol, it is usually sufficient to detect the presence of the principal component

[1] Journal f. prakt. Chemie, Neue Folge 18 (1878), 282.

[2] E. Baumann: Formation of Phenol during the Putrefaction of Proteins. Ber. d. Deutsch. chem. Ges. 10 (1877), 685; Zeitschr. f. physiol. Chem. 1 (1877), 61.

[3] L. Brieger: Aromatic Products of the Putrefaction of Albumin. Zeitschr. f. physiol. Chem. 3 (1879), 139.

of lysol, that is, the cresols. This is accomplished by strongly acidifying the material with tartaric or dilute sulphuric acid and distilling with steam. The cresols pass over almost quantitatively. They may be separated from the hydrocarbons that distil over at the same time by extracting the distillate with ether. The residue left upon evaporating the ether solution is shaken with dilute sodium hydroxide solution and petroleum ether. The latter dissolves only the hydrocarbons. The cresols may be precipitated from the alkaline aqueous solution by dilute hydrochloric or sulphuric acid. They are finally extracted with ether and the solution in this solvent is evaporated. A saturated aqueous solution of the ether residue may be used for the following general phenol tests:

1. The colors given by the three cresols with dilute ferric chloride solution are different. The color with o-cresol is blue changing in a few minutes to green; with m-cresol evanescent blue changing to a dirty yellowish brown with turbidity; and with p-cresol a permanent blue-violet.

2. Only o-cresol gives a positive test with Melzer's benzaldehyde test. Consequently by this reaction it is possible to determine whether o-cresol is present in crude coal-tar creosote or in lysol (*Liquor Cresoli Saponatus*) prepared from it.

3. All three cresols give a positive test with Millon's reagent.

4. Lex's hypochlorite test gives first a green and then a blue color with o- and m-cresol; and with p-cresol usually only a dirty greenish color.

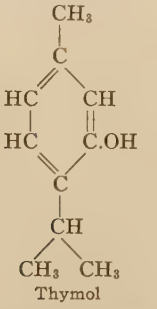
Examination of Urine in Lysol Poisoning

In suspected lysol poisoning, as in carbolic acid poisoning, the urine is first examined chemically and microscopically. According to Fries,[1] lysol poisoning causes severe injury to the kidneys recognizable by excessive elimination of erythrocytes, pigment- and epithelial-casts. Moreover masses of kidney epithelial casts, red and white blood-corpuscles, and much albumin are present. These casts were 3.5 millimeters in length and could be seen with the naked eye as red-brown structures on the bottom of the urine-glass. In lysol poisoning both conjugated aromatic sulphuric and glycuronic acids of the urine are greatly increased. Consequently the normal ratio between conjugated sulphuric acid and sulphate-sulphuric acid is altered in favor of the former.

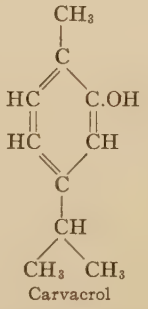
[1] Fries: Münchener med. Wochenschr. 1904, 709.

THYMOL

The two isomeric methyl-isopropyl-phenols, thymol and carvacrol, $C_6H_3(CH_3)(C_3H_7)OH$, differ from each other, as may be seen from their structural formulae:



Thymol



Carvacrol

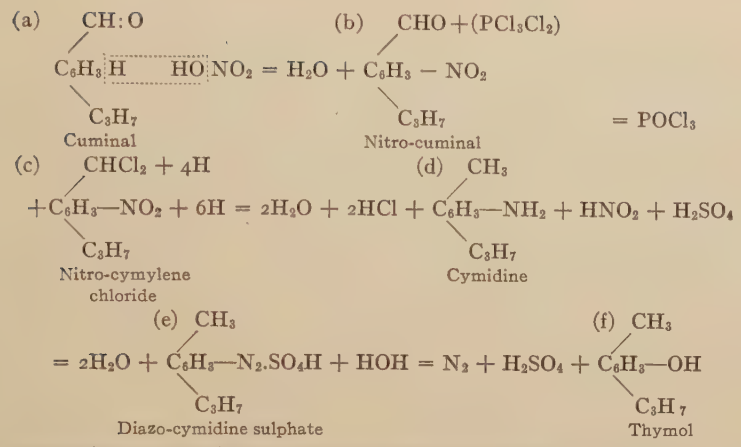
Thymol is 1,4,3- and carvacrol 1,4,2-methyl-isopropyl-phenol.

Thymol is a component of different essential oils. Together with cymene, $C_{10}H_{14}$, and thymene, $C_{10}H_{16}$, it occurs to the extent of 20–50 per cent. in essential oil of Thymus vulgaris; 50–60 per cent. in the North American labiate, Monarda punctata; about 40 per cent. in the Japanese labiate, Mosula japonica; and 50–60 per cent. in the oil from the fruit of the East Indian umbellifera, Ptychotis ajowan. Together with a small quantity of carvacrol, thymol also occurs in the oil of Thymus Serpyllum.

Isolation.—Shake the given essential oil with warm 30 per cent. sodium hydroxide solution (sp. gr. 1.330) and after several hours dilute the mixture with three times its volume of hot water. Separate the precipitated hydrocarbons from the aqueous solution of sodium thymolate, $C_6H_3(CH_3)(C_3H_7)ONa$, and decompose the latter with hydrochloric acid. Draw off the thymol which separates as an oil, free it from water, distil and set the distillate in a cool place to crystallize. Thymol may also be recrystallized from glacial acetic acid.

Synthesis of Thymol.—This may be brought about by starting with cuminal.[1] Cuminal, or isopropyl-benzaldehyde, $(CH_3)_2CH.C_6H_4.CHO$, is purified by means of its sodium bisulphite compound. The product thus obtained is then converted into nitro-cuminal by treating it with a mixture of fuming nitric acid and concentrated sulphuric acid with cooling. The aldehyde oxygen in this compound is replaced by chlorine through the agency of phosphorus pentachloride and the resulting nitro-cymylene chloride is then reduced to cymidine by means of zinc and hydrochloric acid. In the form of its sulphate the latter is diazotized in the usual manner in sulphuric acid solution with sodium nitrite. Diazotized cymidine by boiling with water is converted into thymol which is finally obtained pure by distillation with steam:

[1] O. Widmann: Synthesis of Thymol from Cuminal. Ber. d. Deutsch. chem. Ges. 15 (1888), 166.



Properties.—Thymol forms large, colorless, hexagonal crystals that melt at 50–51°, boil at 230° and have the odor of thyme. Thymol crystals (sp. gr. 1.028) sink in water, whereas melted thymol floats on water. Even at 100° thymol vaporizes rather rapidly and consequently distils easily with steam. When fused or distilled, thymol frequently remains liquid for some time. Thymol dissolves in about 1200 parts of water and in less than 1 part of alcohol, ether and chloroform. It is readily soluble in solutions of caustic alkalies.

Use and Physiological Behavior.—Thymol acts as a preventive of fermentation and putrefaction but its strength as an antiseptic is less than that of carbolic acid and salicylic acid. It is an excellent intestinal disinfectant but the fatty acid esters of thymol, such as thymol palmitate, $C_{15}H_{31}.COOC_6H_3(C_3H_7)(CH_3)$, are more active and at the same time less toxic than thymol itself. For external use thymol serves as a substitute for carbolic acid in the treatment of wounds and also in chronic skin diseases. It is a component of many lotions used for the mouth and teeth. As a rule the usual medicinal doses of thymol, aside from local irritations, produce no dangerous secondary effects. In the case of certain individuals ringing in the ears, headache and violent delirium have been observed. Even collapse accompanied by paralysis and somnolence appeared. Larger doses of thymol have a paralyzing action upon animals. The post-mortem appearances in such cases, as a result of the action of thymol, are fatty liver, and irritation of the kidneys and lungs (Kobert). Like phenol, after previous conjugation, thymol is eliminated in the urine as thymol-sulphuric acid, thymol-glycuronic acid, and after oxidation partly as thymo-quinol-sulphuric acid. In thymol-urine there is the further occurrence of the chromogen of a green pigment that becomes indigo-blue when heated with hydrochloric acid and purple-red on the subsequent addition of ammonia. This blue dyestuff can be extracted with chloroform. (Blum.[1]) The phenol-glycuronic acid of thymol-urine can be best detected in the form of the water insoluble dichloro-thymol-glycuronic acid, $C_6HCl_2(CH_3)(C_3H_7).O.CH(OH).(CH.OH)_4.COOH$ (?). To produce this compound, concentrated hydrochloric acid and sodium hypochlorite solution are added

[1] F. Blum: Thymol-Glycuronic Acid. Zeitschr. f. physiolog. Chemie 16 (1892), 514.

to the usually green colored thymol-urine until the green color has disappeared. After rather long standing, within 4 days, long crystalline needles separate and are dissolved in sodium carbonate solution. After extracting this solution with ether, the conjugated dichloro-glycuronic acid may be precipitated by dilute sulphuric acid in fine white needles. This compound is a monobasic acid having the formula, $C_{16}H_{22}Cl_2O_8$, and melting at 125–126°. It is insoluble in cold water but easily soluble in alcohol, ether, acetone and benzene. As a phenol it is also readily soluble in solutions of caustic alkalies. This acid is laevo-rotatory; α-D-66° 11′ for an alcoholic solution. It reduces neither Fehling's nor ammoniacal silver nitrate solution. When boiled with 5 per cent. sulphuric acid, it undergoes cleavage into glycuronic acid and 1, 6-dichloro-thymol. In the latter compound the two chlorine atoms are in the ortho-position to the methyl group.

Detection of Thymol

To detect thymol with greater certainty, acidify the material first with tartaric or dilute sulphuric acid and then distil. Thymol passes over rather easily with steam and usually separates in the distillate in the form of oil-drops, since it dissolves in water only with very great difficulty (1:1200). Then extract the distillate, which may have the thyme-like odor of thymol, with ether, evaporate or distil the ether extract, and test the residue for thymol by the following reactions:

1. Concentrated sulphuric acid dissolves thymol, giving in the cold a colorless or faintly yellowish solution that takes on a beautiful rose-red color when gently warmed.

2. Dissolve a small crystal of thymol in 1 cc. of glacial acetic acid and carefully pour this solution upon 6 drops of concentrated sulphuric acid and 1 drop of officinal nitric acid (25 per cent.). The last two acids are at the bottom of the test-tube and a blue-green zone appears at the contact-surface of the two liquids. If the contents of the tube are shaken around, the mixture appears blue-green by reflected light and red-violet by transmitted light. The mixture is fluorescent.

3. **Piria's Test.**—Neither the aqueous nor the alcoholic solution of thymol gives a color with ferric chloride. But if a solution of thymol in concentrated sulphuric acid (see above) is poured into 10 volumes of water and the mixture is allowed to stand with frequent shaking at 30–35° with an excess of white lead, the neutral filtrate gives a beautiful violet color with a little ferric chloride solution.

4. **Eykmann's Test.**—If a few drops of Eykmann's reagent are added as an upper layer to a solution of thymol in concentrated sulphuric acid, a red zone appears. Then if the contents of the tube

are well-cooled and mixed, the mixture first turns red, then immediately green. Upon dilution with water the red color reappears, changing to yellow upon subsequent addition of ammonia.

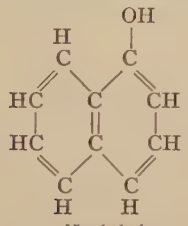
5. Liebermann's Test.—Liebermann's reagent colors a solution of thymol in concentrated sulphuric acid first red and then almost immediately green.

6. Lustgarten's Test.—If a solution of thymol in a little 50 per cent. potassium hydroxide solution is only gently warmed and a few drops of chloroform are added, the mixture will have a beautiful violet-red color.

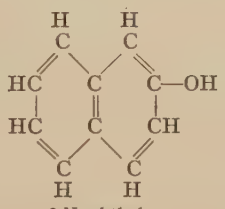
In an aqueous solution of thymol, bromine water produces only a turbidity but gives no crystalline precipitate (distinction from carbolic acid). An aqueous thymol solution does not give with Millon's reagent the red color so characteristic of monacid phenols.

NAPHTHOLS

As in the case of all mono-substitution products of naphthalene, there are also two isomeric monoxy-naphthalenes, $C_{10}H_7.OH$, α- and β-naphthol:



α-Naphthol



β-Naphthol

Preparation.—Both naphthols are prepared by fusing the sodium salts of the corresponding naphthalene sulphonic acids with sodium hydroxide, dissolving the melts in water, adding hydrochloric acid in excess to the solutions, and recrystallizing the precipitated naphthols from boiling water, after first washing with water, or purifying by sublimation:

$$(\alpha)\ C_{10}H_7.SO_2.ONa + 2NaOH = C_{10}H_7.ONa + Na_2SO_3 + H_2O,$$
$$(\beta)\qquad C_{10}H_7.ONa + HCl = C_{10}H_7.OH + NaCl.$$

α-Naphthol.—This compound crystallizes in colorless, shining, silky needles that melt at 95° and have a characteristic odor. It sublimes with gentle heat and is also volatile with steam. It is only slightly soluble in water but readily soluble in alcohol, ether and chloroform.

β-Naphthol.—This compound crystallizes in white, shining, rhombic leaflets melting at 122°. It easily sublimes when more strongly heated and is also volatile with steam. It dissolves in 1000 parts of cold and in 75 parts of hot water, and is readily soluble in alcohol, ether and chloroform. Solutions of β-naphthol in sodium or potassium hydroxide and ammonia exhibit blue fluorescence

Medicinal Use and Toxic Action of β-Naphthol

In the form of a 10–15 per cent. ointment, β-naphthol is used principally in skin diseases. Naturally in rubbing it over the entire body abundant absorption takes place. Lewin, who had a patient with itch rubbed with 15 grams of β-naphthol, observed the appearance of eczema followed after 14 days by acute nephritis with albuminuria. On the other hand, an 8 year old and a 6 year old boy were treated for scabies with 2 per cent. β-naphthol ointment, the elder receiving in all 4 grams and the younger 3 grams of the ointment. After 20 days both became ill from acute nephritis accompanied by oedema. The younger died and the post-mortem confirmed the diagnosis. Consequently in addition to severe irritation of the skin and mucous surfaces, β-naphthol after absorption may also give rise to acute effects in the blood, central nervous system, and especially in the kidneys (Kobert).

Behavior of β-Naphthol and Naphthalene in Organism

According to Edlefsen,[1] naphthalene in doses of 0.5–0.75 gram is oxidized in the organism to β-naphthol which is then eliminated in the urine mostly as conjugated glycuronic acid and in smaller quantity as ethereal sulphuric acid. After administration of small doses of β-naphthol, 0.3–0.5 gram, β-naphthol-glycuronic acid is only exceptionally found in the urine, since most of the β-naphthol that gets into the circulation through absorption is eliminated as ethereal sulphuric acid. Benzo-naphthol, or β-naphthol-benzoate, $C_6H_5.COOC_{10}H_7$, administered in small and medium doses of 0.6, 0.9 or 1.2 grams, is almost always eliminated as ethereal sulphuric acid. To bring about the hydrolytic cleavage of the latter and detect β-naphthol, the given urine is boiled for some time under a reflux with 10 cc. of concentrated hydrochloric acid. The β-naphthol is then extracted with ether and the ether solution distilled or evaporated. The residue that remains can then be tested for β-naphthol by the reactions given below. To test urine direct for β-naphthol, add according to Edlefsen to 5–10 cc. of urine 3–6 drops each of calcium hypochlorite solution and hydrochloric acid. Owing to the formation of naphthoquinone the color of the urine becomes lemon-yellow. Extract this dyestuff with ether and add some of the ether extract to 1 per cent. aqueous resorcinol solution. A red ring will appear. A β-naphthol-urine also shows blue fluorescence upon addition of sodium or potassium hydroxide solution or ammonia.

Detection of α- and β-Naphthol

1. Calcium hypochlorite solution gives a violet color with an aqueous α-naphthol solution, but is without effect upon a solution of β-naphthol.

2. Chlorine water produces a white precipitate with an aqueous α-naphthol solution. This precipitate dissolves in ammonia with a bluish color. Under the same conditions β-naphthol produces a white turbidity that disappears with an evanescent green color upon addition of ammonia, the solution becoming colorless.

[1] G. Edlefsen: Experiments upon Elimination and Detection of β-Naphthol in Urine after Administration of Small Doses of Naphthalene, Benzo-Naphthol and β-Naphthol. Archiv f. experim. Pathol. u. Pharmakol. 82 (1905), 429.

3. Ferric chloride solution added to an aqueous solution of α-naphthol gives a white precipitate of α-dinaphthol, $C_{20}H_{12}(OH)_2$, that soon turns violet, whereas aqueous β-naphthol solution takes on a greenish color and gradually white flocks of β-dinaphthol separate.

4. **Piria's Test.**—Warm α-naphthol at 80–100° for some time upon the water-bath with four times the quantity of concentrated sulphuric acid. Dilute this solution with about 10 times the quantity of water and neutralize with white lead. Ferric chloride solution will impart a beautiful green color to the filtrate, whereas β-naphthol gives a violet or beautiful blue color.

5. **Chloral Hydrate Test.**—Warm α-naphthol with 20 times the quantity of chloral hydrate upon the water-bath for 10 minutes. The mixture has a ruby-red color and the product of the reaction is soluble in alcohol with a red color. Under the same conditions β-naphthol gives a mixture having a deep blue color and soluble in alcohol with blue color.

6. **Formaldehyde Test.**—Dissolve 0.1 gram of α-naphthol in a little sodium hydroxide solution and warm after adding a few drops of formaldehyde solution. The color of the mixture is green and soon changes to blue. This test is given neither by β-naphthol nor by a mixture of the two isomeric naphthols.

7. **Lustgarten's Test.**—The solution of α-naphthol in a little concentrated potassium hydroxide solution gives a blue-violet color when warmed with a few drops of chloroform. A pure blue color is given by β-naphthol.

8. **Eykmann's Test.**—A black-green ring is given by α-naphthol, whereas a beautiful green fluorescing color-zone appears with β-naphthol.

9. **Millon's Test.**—Even in the cold, α-naphthol gives a beautiful scarlet-red color, whereas β-naphthol produces more of a reddish brown color with separation of a flocculent precipitate.

10. **Diazo-Reaction.**—α-Naphthol gives an intense purple-red and β-naphthol a beautiful scarlet-red color.

11. **Jorissen's Test.**—Addition of about 5 cc. of 0.1 n-iodine solution and an excess of sodium hydroxide solution to 0.1 gram of α-naphthol produces a turbid, intense violet solution, but β-naphthol gives a clear colorless solution.

12. **Reverse Molisch's Test.**—Add as an upper layer to concentrated sulphuric acid a few drops of an alcoholic solution of α-naphthol containing very dilute sugar solution. A violet ring will appear. If the entire mixture is shaken while cooling, it will become violet- to purple-red. This test is not given by β-naphthol.

Isolation of the Two Naphthols

To separate either or both of these two naphthols from material, for example, from urine, acidify it with dilute sulphuric acid and distil. Extract the distillate with ether, evaporate or distil off the ether, and examine the residue for α- or β-naphthol. If the residue is not too small, it may be purified by dissolving in alcohol, heating the solution with bone-black, and evaporating the filtrate.

CHLOROFORM

Chloroform distils easily with steam and appears in the first fraction in largest quantity. When much chloroform is present, it will

separate from the distillate as heavy, colorless globules, whereas a small quantity will remain in solution. This solution usually has the characteristic odor and sweetish taste of chloroform. In a fatal case of suspected chloroform poisoning, the detection of this compound, especially in blood, brain, and also in urine, should be undertaken as soon as possible, since chloroform on account of its great volatility may completely and easily disappear from the body. Chloroform may be detected in the distillate by the following tests:

1. **Hofmann's Phenyl-isocyanide Test.**—Add 1–2 drops of aniline to the chloroform solution and then a few cc. of aqueous or alcoholic potassium hydroxide solution. Gentle heat will produce phenyl-isocyanide (C_6H_4NC). The penetrating and repulsive odor of this compound is easily recognized. This is a very delicate test by which 1 part of chloroform dissolved in 6000 parts of alcohol may be detected with certainty (A. W. Hofmann):

$$CHCl_3 + C_6H_5.NH_2 + 3KOH = C_6H_5.NC + 3KCl + 3H_2O.$$

Notes.—Chloral, chloral hydrate, bromal, bromoform, iodoform and tetrachloro-methane also give this test.

The fact that aniline boiled with potassium hydroxide solution gives a peculiar faintly ammoniacal odor, even when chloroform is absent, must not be overlooked. There is small chance, however, of confusing this odor with the repulsive smell of phenyl-isocyanide. In doubtful cases warm some water, containing a drop of aniline and a trace of chloroform, with potassium hydroxide solution and compare the odor with that in question.

2. **Schwarz's[1] Resorcinol Test.**—Dissolve about 0.1 gram of resorcinol in 2 cc. of water, add a few drops of sodium hydroxide solution, and finally the liquid containing chloroform. This mixture heated to boiling will develop even in very dilute solution a yellowish red color attended by a beautiful yellowish green fluorescence.

Chloral, bromal, bromoform and iodoform also give this test.

3. **Lustgarten's[2] Naphthol Test.**—Dissolve a few centigrams of α- or β-naphthol in 1–2 cc. of 33 per cent. aqueous potassium hydroxide solution. Warm to 50° and add the solution to be tested. If chloroform is present, the mixture will become blue or blue-green. With β-naphthol the color is less stable, changing in the air first to green and then to brown. Acidification of the blue liquid produces

[1] Schwarz: Fresenius Zeitschr. f. analyt. Chem. 27, 668.

[2] S. Lustgarten: Detection of Chloroform, Iodoform and Naphthol in Animal Liquids and Organs. Monatsh. f. Chem. 3 (1882), 715.

a brick-red precipitate, consisting of a mixture of naphthol and a red dyestuff.

Chloral, chloral hydrate, bromal, bromoform and iodoform also give this test.

4. Fujiwara's[1] Pyridine Test.—Mix 2 cc. of pyridine with 3 cc. of 10 per cent. sodium hydroxide solution, heat to boiling and add 1 cc. of the liquid to be tested. Even a trace of chloroform will produce a bright, blue-red color.

Chloral, bromoform, iodoform and several similar compounds also respond to this test.

One part of chloroform in 1,000,000 parts of water, 500,000 parts of ether, or 300,000 parts of alcohol can be detected by this test. It is equally sensitive toward the other substances mentioned.

5. Reduction Tests.—(a) **With Fehling's Solution.**—Warm the liquid containing chloroform with Fehling's solution. A red precipitate of cuprous oxide will appear.

(b) **With Ammoniacal Silver Nitrate Solution.**—Add excess of ammonium hydroxide to silver nitrate solution and then the liquid containing chloroform. Heat will produce a black precipitate of metallic silver.

These reactions are not characteristic of chloroform, because many volatile organic substances, such as formic acid and aldehydes, that may occur in distillates from animal material, reduce Fehling's and ammoniacal silver nitrate solution.

6. Cyanide Test.—This test is based upon the conversion of chloroform into ammonium cyanide when heated with alcoholic ammonia:

$$CHCl_3 + 5NH_3 = H_4N.CN + 3(H_4N)Cl.$$

Seal the liquid to be tested for chloroform in a glass tube (pressure-tube[2]) with a little solid ammonium chloride and alcoholic potassium hydroxide solution. Not more than a quarter of the tube should be filled with liquid. Heat for several hours in a boiling water-bath. Cool the tube, remove the solution and test for hydrocyanic acid by the Prussian blue reaction. A positive test means that the distillate contained chloroform, provided that preformed hydrocyanic acid was not present in the material.

[1] Chemical Abstracts 11 (1917), 3201. Sitzungsb. u. Abhandl. Natur fors. Ges. Rostock, 6 (1914), 1. See also J. H. Ross: J. Biol. Chem. 58 (1923–24), 641.

[2] An ordinary citrate of magnesium bottle is a convenient apparatus for this test. Wrap a towel around the bottle, place it in the water-bath, and gradually raise the temperature to boiling. Do not remove the bottle until it is cold. Tr.

Estimation of Chloroform in Parts of Cadavers

Mix a weighed portion of material with water containing tartaric acid and distil as long as there is any chloroform. To determine when this point is reached, apply the phenyl-isocyanide test to a few cc. of liquid collected at the end of distillation. Add some calcium carbonate to combine with free hydrochloric acid. Warm the distillate to about 60° and draw washed air through it by suction. Pass the air through a combustion-tube heated at a high temperature and then into silver nitrate solution acidified with nitric acid. Weigh the precipitated AgCl (N).

Calculation.

$$3AgCl : CHCl_3 = N : x$$

This method is based upon the fact that chloroform heated with steam above 200° is decomposed into carbon monoxide, hydrochloric and formic acid:

$$(\alpha)\ CHCl_3 + H_2O = CO + 3HCl,$$
$$(\beta)\ CHCl_3 + 2H_2O = H.COOH + 3HCl.$$

In a series of blank experiments B. Fischer[1] has shown that the stomach, stomach-contents and blood of a person, who has not taken chloroform, give no volatile chlorine compounds under these conditions. By this method B. Fischer found in the cadaver of a laborer, who had died during chloroform narcosis, the following quantities of chloroform:

Weight	Organ	Chloroform
985 grams	Stomach and contents and parts of the intestine	0.1 gram
780 grams	Lungs and blood from the heart	0.055 gram
445 grams	Portions of spleen, kidneys and liver	traces
480 grams	Brain	0.07 gram

From these results it appears that most of the chloroform was in the brain and blood.

Quantitative Estimation of Chloroform by Fujiwara's Test

Cole[2] has recently applied the pyridine test for chloroform, described by Fujiwara and Ross,[3] to the quantitative estimation of small amounts of chloroform either in aqueous solutions, or in acidified (0.1 per cent. HCl) extracts of animal tissues.

[1] B. Fischer: Jahresber. d. chem. Untersuchungsamtes d. Stadt Breslau f. d. Zeit from April 1, 1894, until March 31, 1895.

[2] W. H. Cole: The Pyridine Test as a Quantitative Method for the Estimation of Minute Amounts of Chloroform. Jour. of Biolog. Chem. 71 (1926), 173.

[3] J. H. Ross: J. Biol. Chem. 58 (1923–24), 641.

Procedure.—Measure from a burette into a narrow test-tube (10 cc. capacity) 2 cc. of 20 per cent. sodium hydroxide solution. Add also from a burette 1 cc. of chemically pure pyridine (colorless) and from a 1 cc. pipette 1 cc. of the solution to be tested. Loosely stopper the test-tube to prevent evaporation of pyridine, shake constantly, and immerse for 1 minute in water at 100° well above the liquid level. Then immerse in cold water and reduce the temperature to 20°. The pyridine and test solution, colored pink or red if chloroform is present, rise above the alkali during cooling. Transfer the former by means of a 1 cc. pipette to a color comparison tube and compare the solution in a comparator with color standards previously made up to match the colors obtained from known concentrations of chloroform.

In quantitative work it is essential that the amounts of pyridine and test-solution be measured accurately, since the color produced is a function of the volume over which it is distributed. Since pyridine is volatile (Bpt. 115°), long, loosely stoppered tubes are necessary to keep the volume of pyridine as constant as possible. The color comparison tubes must be of the same diameter and of the same kind of glass as that used for the color standards.

Cole has found that chloroform in concentrations from 0.1 per cent. (by volume at 15°) down to 0.0001 per cent. (1 part of chloroform in one million parts of water) may easily be detected. In his original paper the author gives further details with regard to the preparation of color standards, as well as certain precautions that should be observed in carrying on quantitative estimations by this method.

Behavior of Chloroform in Human Organism

During chloroform narcosis, this anaesthetic when inhaled first passes from the air into the blood-plasma which then transmits it to the red blood-corpuscles where it may accumulate in relatively large quantity. Air passed through blood will remove chloroform completely. Pohl (see Kobert's "Intoxikationen") states that blood may contain 0.62 per cent. of chloroform, three-fourths of which will be in the red blood-corpuscles. At the height of a harmless narcosis the blood contained only 0.035 per cent. of chloroform. Absorption of chloroform takes place from all parts of the body. The stimulative action of chloroform upon the mucous membranes of the respiratory passages explains such disturbances as coughing, secretion of saliva and reflex slowing of respiration and heart-beat, occurring at the beginning of narcosis. Dilatation of the blood-vessels of organs living after death is due to paralysis caused by even small doses of chloroform. A drop in blood-pressure accompanies paralysis of the brain and the heart's action is feebler and slower. Several researches with regard to the effect of inhaled chloroform upon human and animal metabolism have shown an increase in the quantity of nitrogen in the urine after prolonged narcosis because more protein is decomposed. The amount of neutral sulphur and chlorine in the urine also increases. The increase of the latter is due in part at least to the conversion of chloroform into chloride. The acidity of the urine is also much higher. A final charateristic of chloroform urine is the high content of reducing substances. The increased protein decomposition in chloroform narcosis affects both reserve protein and that of the tissues. This may explain degeneration in red blood-corpuscles, glandular organs, the heart, etc., after frequent narcoses or one of long duration.

The temporary or permanent paralysis of isolated animal or vegetable cells, such as leucocytes, ciliated cells, yeast cells, algae and spores, is evidence of the antiseptic action of chloroform when present in proper concentration in air or in a liquid. This explains the use of 1 per cent. aqueous chloroform solution as an antiseptic. Added to urine it acts as a preservative. Therefore it may be used in the study of the action of enzymes but not of bacteria, though all micro-organisms are not paralyzed or killed by chloroform water.

Distribution of Chloroform in Cadaver

A large part of the absorbed chloroform undergoes decomposition in the body and a part is eliminated by the lungs. Pohl and Hans Meyer have studied the distribution of chloroform in the body and have found that the red blood-corpuscles and the brain are most likely to show this poison. After chloroform has been inhaled, some will appear in the gastric juice but at most only traces in the urine. In but two out of 15 cases of chloroform narcosis was this poison found in the urine and then only in traces.

Kobert states that as a rule it is the exception to find chloroform itself in the cadaver, because part of the poison is converted into chloride in the human organism and part is quickly exhaled during respiration. Usually it is possible to detect chloroform in the breath of patients even 24 hours after narcosis. Büdinger states that the mucus of the respiratory passages retains chloroform.

CHLORAL HYDRATE

Chloral hydrate, $CCl_3.CH(OH)_2$, forms transparent crystals that are dry, permanent and colorless. It dissolves with ease in water, alcohol and ether, and less easily in chloroform, carbon disulphide and fatty oils. Chloral hydrate has a pungent odor and a faintly bitter taste. From an aqueous solution acidified with tartaric acid it distils very slowly with steam. Therefore the complete distillation of a large quantity of chloral hydrate requires considerable time. Chloral hydrate appears as such in the distillate. If the reaction of the material is alkaline during distillation, the distillate will not contain chloral hydrate but chloroform as a resulting product of its decomposition.

Preparation.—Pass dry chlorine into 96 per cent. alcohol until the evolution of hydrogen chloride diminishes. At the outset the alcohol should be cooled but toward the end of the process a temperature of about 60–70° is advisable. When the action of chlorine upon the alcohol is complete, the reaction-product will be a crystalline mass when cold. In the main it will consist of chloral-alcoholate and some chloral hydrate (see below). To prepare pure chloral, warm this crude product with about the same volume of concentrated sulphuric acid in a flask under a reflux as long as hydrogen chloride is given off. The chloral that separates is withdrawn and purified by distillation. It boils at 96–97°. Chloral hydrate is formed with considerable evolution of heat by mixing 100 parts

of chloral with 12 parts of water. If this mixture of chloral and water warmed to about 50° is mixed with about half its volume of warm chloroform, chloral hydrate upon cooling will separate in loose crystals. These recrystallized from warm chloroform, carbon disulphide or petroleum benzine will give well-formed crystals.

Explanation.—In the first phase of the reaction chlorine converts alcohol into acetic aldehyde. The latter then in presence of more alcohol (2 molecules) may condense forming acetal. Chlorine converts acetal into trichlor-acetal, the final substitution-product, from which under the influence of hydrogen chloride trichlor-alcoholate and ethyl chloride are produced. Then trichlor-alcoholate warmed with concentrated sulphuric acid yields chloral which finally combines with water forming chloral hydrate:

(a) $CH_3.CH_2.OH + Cl_2 = 2HCl + CH_3.CH{:}O$ (Acetic aldehyde),
(b) $CH_3.CH{:}O + 2CH_3.CH_2.OH = H_2O + CH_3.CH(OC_2H_5)_2$ (Acetal),
(c) $CH_3.CH(OC_2H_5)_2 + 3Cl_2 = 3HCl + CCl_3.CH(OC_2H_5)_2$ (Trichlor-acetal),
(d) $CCl_3.CH(OC_2H_5)_2 + HCl = C_2H_5.Cl + CCl_3.CH(OC_2H_5)(OH)$ (Trichlor-alcoholate),
(e) $CCl_3.CH(OC_2H_5)(OH) + H_2SO_4 = (C_2H_5)SO_4H + H_2O + CCl_3.CH{:}O$ (Chloral),
(f) $CCl_3.CH{:}O + H_2O = CCl_3.CH(OH)_2$ (Chloral hydrate).

The action of chlorine upon alcohol is expressed, though not entirely, by equations a–d, for unquestionably secondary reactions take place accounting for the formation of ethylidene chloride, $CH_3.CHCl_2$, methyl-chloroform, $CCl_3.CH_3$, trichlor-acetic acid, $CCl_3.COOH$, and other chlorinated products.

Behavior of Chloral Hydrate in the Animal Body

Because of the ease with which chloral hydrate undergoes decomposition, tests for its presence in the cadaver should be made as soon as possible after death, for after putrefactive processes have set in the chances of finding it are not very promising. Stomach-contents and vomitus are especially suitable material for its detection. Chloral hydrate probably undergoes change in the liver. As a result after 2 hours, but sometimes not until 18 hours have elapsed, the urine[1] contains urochloralic acid, as well as a trace of unaltered chloral hydrate, the latter probably appearing only after very large doses. Consequently blood and urine seldom contain more than traces of chloral hydrate. Detection of urochloralic acid in the urine therefore is probably the most reliable means of reaching a decision with regard to suspected chloral poisoning.

Detection of Chloral Hydrate

Chloral hydrate like chloroform will give the phenyl-isocyanide, resorcinol, naphthol and pyridine tests. But the distillate containing chloral hydrate does not have the characteristic chloroform odor which is also scarcely perceptible in very dilute aqueous chloroform

[1] The method of detecting urochloralic acid in chloral urine is taken up in Chapter V, page 555, of this book.

solutions. In distinction from chloroform, chloral hydrate gives the aldehyde-reaction with Nessler's reagent. Add a few drops of this reagent to an aqueous chloral hydrate solution and shake. It will produce a yellowish red precipitate, the color of which will change after a while to a dirty yellowish green.

When the quantity of chloral hydrate is not too small, it may also be detected by the following procedure. Heat a portion of the distillate for 30 minutes under a reflux with calcined magnesium oxide upon a boiling water-bath. Magnesium formate and chloroform are produced by decomposition of chloral hydrate:

$$2CCl_3.CH(OH)_2 + MgO = 2CHCl_3 + Mg(OOCH)_2 + H_2O.$$

Both products of this reaction, chloroform and formic acid, may be detected by distilling off a few cc. of the solution and testing for chloroform by the phenyl-isocyanide, resorcinol, naphthol and pyridine reactions. Filter the residue from distillation, concentrate the filtrate to a few cc. by evaporation and divide into two portions to test for formic acid. Warm one portion with a few drops of mercuric chloride solution which will give a white precipitate of mercurous chloride, if formic acid is present; and heat the other with a little silver nitrate solution which will give a black precipitate of metallic silver if the filtrate contained formic acid. The following equations explain these two tests:

(a) $4HgCl_2 + Mg(COOH)_2 = 4HgCl + 2HCl + 2CO_2 + MgCl_2.$
(b) $4AgNO_3 + Mg(COOH)_2 = 4Ag + 2HNO_3 + 2CO_2 + Mg(NO_3)_2.$

Quantitative Estimation of Chloral Hydrate

(a) The following method gives satisfactory results, if pure aqueous chloral hydrate solutions are used and the excess of potassium hydroxide solution taken is titrated at once. Under these conditions chloral hydrate is decomposed in the following manner:

$$CCl_3.CH(OH)_2 + KOH = CHCl_3 + H.COOK + H_2O.$$

Procedure.—Shake the aqueous chloral hydrate solution well for a few minutes with an excess of n-potassium hydroxide solution, add phenol-phthalein as indicator, and at once titrate the excess of alkali with n-acid.

Calculation.—According to the above equation, 1000 cc. of n-alkali correspond to 1 gram-molecule = 165.38 grams of chloral hydrate.

(b) In dealing with a distillate from any material of known amount, heat it under a reflux for 5–6 hours with an excess of chlorine-

free, alcoholic potassium hydroxide and then evaporate upon the water-bath. Dissolve the residue in water, acidify with dilute nitric acid, precipitate chlorine with silver nitrate, and weigh as silver chloride. Under these conditions alcoholic potassium hydroxide solution converts all chlorine, first separated as chloroform, into potassium chloride:

(a) $CHCl_3 + 4KOH = 3KCl + H.COOK + 2H_2O$,
(b) $CHCl_3 + 3KOH = 3KCl + CO + H_2O$.

Consequently 3 molecules of silver chloride (430.14) correspond to 1 molecule of chloral hydrate (165.38). Obviously this method of estimation is applicable only when the distillate, in which chloral hydrate is to be determined, contains neither hydrochloric nor hydrocyanic acid.

Quantitative Estimation of Chloral Hydrate in Blood and Tissues

(Archangelsky[1])

Distil the material for 12–20 hours with its own weight of 20 per cent. phosphoric acid, repeating the process if the distillate is turbid or yellow. To complete the decomposition of chloral hydrate into chloroform and formic acid, add 50 cc. of sodium hydroxide solution to the distillate and concentrate upon the water-bath to about 20 cc. Neutralize the solution exactly and heat for 6 hours upon the water-bath with an excess of mercuric chloride solution. Finally weigh the precipitated mercurous chloride. Satisfactory results were obtained by this method when known quantities of chloral hydrate were added to blood and organs. Using this method, Archangelsky has shown that chloral hydrate is not uniformly distributed in the blood but is contained especially in the blood-corpuscles. When narcosis begins, there is less chloral hydrate in the brain than in the blood. But later the percentage of the poison in the brain is higher than in the blood. Archangelsky has further shown how much chloral hydrate the blood must contain before narcosis can appear. A dog's blood must contain 0.03–0.05 per cent. When the blood contains 0.12 per cent., respiration ceases.

Butyl-Chloral Hydrate.—This compound which has the formula, $CH_3.CHCl.CCl_2.CH(OH)_2$, has a limited use in medicine as a hypnotic and anti-neuralgic. It melts at 78° and forms white leaflets having an aromatic odor and bitter taste. It is rather difficultly soluble in cold water but dissolves readily in alcohol and ether. Since it is easily volatile with steam, it passes into the distillate when material is faintly acidified and distilled. With caustic alkalies it yields no chloroform and so does not give the phenyl-isocyanide test. This behavior serves to differentiate it from chloral hydrate which it otherwise resembles. The products formed as a result of decomposition by alkalies are dichloro-propylene, hydrochloric acid and formic acid. On the other hand, it reacts with Nessler's reagent like chloral hydrate, forming a red-brown precipitate.

[1] C. Archangelsky: Distribution of Chloral Hydrate and Acetone in the Organism. Arch. f. exper. Path. u. Pharm. 46 (1901), 347.

Butyl-chloral hydrate appears in urine as urobutyl-chloralic acid which can be isolated from urine by the method given for urochloralic acid. Urobutyl-chloralic acid crystallizes in shining, silky, star-shaped needles, is easily soluble in water, alcohol and ether, turns the plane of polarized light to the left, and reduces Fehling's solution after it has first been boiled with dilute acids. When boiled with dilute mineral acids, urobutyl-chloralic acid takes up water, undergoing cleavage into trichloro-butylalcohol and glycuronic acid:

$$C_{10}H_{15}Cl_3O_7 + H_2O = CH_3.CHCl.CCl_2.CH_2OH + HOC(CHOH)_4COOH.$$

Therefore like urochloralic acid it belongs to the conjugated glycuronic acids. From a physiological point of view, urochloralic and urobutyl-chloralic acid are of especial interest, since the chlorinated constituents of both, trichlor-ethyl alcohol and trichloro-butyl alcohol, arise in the organism from their corresponding aldehydes as a result of a process of reduction; and not until this has taken place can conjugation with glycuronic acid occur.

IODOFORM

Iodoform, CHI_3, crystallizes in shining, hexagonal leaflets or plates. It may also appear as a rather fine crystalline powder, lemon-yellow in color and having a penetrating odor somewhat like saffron. The melting-point of iodoform is approximately 120°. It is nearly insoluble in water; soluble in 70 parts of cold and in about 10 parts of boiling alcohol; and soluble in 10 parts of ether. It is also freely soluble in chloroform. When iodoform is heated violet, vapors of iodine appear.

Detection of Iodoform

Iodoform distils quite easily with steam and gives a milky distillate having a characteristic odor. Extract this distillate with ether and carefully test the residue left after spontaneous evaporation of the solvent. If much iodoform is present, it will form yellow hexagonal plates. To identify iodoform, dissolve this residue, in case it has the odor of this substance, in a little warm absolute alcohol and apply Lustgarten's[1] test:

Lustgarten's Test.—In a rather small, narrow test-tube heat gently over a small flame very little potassium phenolate, that is, a solution of crystallized phenol in concentrated potassium hydroxide solution, with 2–3 drops of the alcoholic solution of the residue. If iodoform is present, a red deposit due to formation of rosolic acid will appear on the bottom of the tube. A few drops of dilute alcohol will dissolve this precipitate with a carmine-red color.

[1] S. Lustgarten: Detection of Chloroform, Iodoform and Naphthol in Animal Fluids and Organs. Monatsh. f. Chem. 3 (1882), 715.

The phenyl-isocyanide, resorcinol and pyridine tests should also be made as described under chloroform (see pages 60 and 61).

Behavior in the Organism.—Too large doses of iodoform, administered externally as well as internally, have repeatedly given rise to fatal poisonings. Stomach-contents, kidneys, brain, blood and urine should especially be examined if poisoning from iodoform is suspected. In one case death ensued within 7 days following the internal administration of 5 grams of iodoform. The decomposition of iodoform gradually takes place in the organism and iodine is slowly eliminated in the urine and saliva and in small quantity also in the faeces. A noteworthy fact observed by Lustgarten is that iodoform cannot be detected either in the urine or blood of dogs poisoned by this compound.

Detection of Iodine in Urine in Iodoform Poisoning

In a suspected case of iodoform poisoning, when this substance can no longer be detected as such, the urine should be examined for iodine. Render about 100 cc. of urine strongly alkaline with sodium cabonate, add 1–2 grams of potassium nitrate, evaporate and heat the residue until it has fused and all particles of carbon have disappeared. On account of the volatility of alkaline iodides, carefully avoid heating the residue too strongly and for too long a time. Add chloroform to an aqueous solution of the cold melt, acidify while cooling with dilute sulphuric acid and shake. The appearance of a violet color in the chloroform obviously cannot be accepted as positive proof that the iodine came from iodoform, since alkaline iodides and many iodoform substitutes containing iodine, when used in the treatment of wounds, contribute iodine to the urine.

NITROBENZENE

Nitrobenzene, $C_6H_5NO_2$, is a yellowish liquid having a high index of refraction and the odor of bitter almonds. The boiling-point at 760 mm. is 209°. In dilute aqueous solution it has a pronounced sweet taste. Nitrobenzene is easily volatile with steam.

Preparation.—Gradually introduce benzene in small quantities into a mixture of concentrated sulphuric and nitric acid (sp. gr. 1.41 = 67.5 per cent.) so that the temperature of the mixture does not exceed 35°. As soon as all the benzene has been added, shake frequently and warm gently for about 1 hour upon the water-bath. Finally pour the product of the reaction into water:

$$C_6H_5 \boxed{H + HO} NO_2 = H_2O + C_6H_5.NO_2.$$

Toxic Action.—Nitrobenzene has a strong poisonous action. Administration of very small quantities of this compound has produced death in human beings. There are records in the literature of several cases where 20 drops, and even 7–8 drops, have caused fatal results. But on the other hand complete recovery has followed poisoning by much larger doses. Fatal poisonings have come also from inhaling nitrobenzene vapor. Within recent years nitrobenzene has been used to some extent as an abortifacient. Nitrobenzene poisons the blood and changes its appearance. It has a chocolate color and at the same time the red blood-corpuscles change their shape and pass into solution. As a result the blood is incapable of uniting with oxygen. The blood of persons poisoned by nitrobenzene is said to contain less than 1 per cent. of oxygen so that death is caused by asphyxiation. Healthy blood contains about 17 per cent. of oxygen by volume. There seems to be no methaemoglobin in blood containing nitrobenzene. Such blood examined spectroscopically shows the two oxyhaemoglobin bands and also a special absorption-band between C and D (Fihlene's nitrobenzene band). It is probable that the slight solubility of this poison necessitates a definite incubation period, for 2–3 hours usually elapse after nitrobenzene has been taken before signs of intoxication appear. A woman, who had taken 10 drops of mirbane oil as an abortifacient, gave no indication of intoxication, that is, unconsciousness and cyanosis, for 8 hours after taking the poison.

Some nitrobenzene passes into the urine. Apparently the organism does not convert it into aniline. In nitrobenzene poisoning human urine contains a brown pigment but only rarely haemoglobin or methaemoglobin. Urine containing nitrobenzene will reduce Fehling's solution. It is also unfermentable and distinctly laevo-rotatory. Possibly it contains a conjugated glycuronic acid.

Behavior of Nitrobenzene in the Cadaver

In a case of nitrobenzene poisoning Rossi[1] showed that the characteristic odor of nitrobenzene can be detected in the cadaver for only a few days. After about 10 days it entirely disappears so that nitrobenzene can no longer be detected as such. Instead of attempting to find nitrobenzene, the analyst should turn his attention to aniline, the reduction product of nitrobenzene.

Detection of Nitrobenzene

In nitrobenzene poisoning the urine and all the organs have the odor of this compound. For the chemical tests the material should first be distilled with water. Nitrobenzene distils quite easily with steam and appears in the distillate as yellowish globules. These are heavier than water and have a characteristic odor. To detect nitrobenzene with greater certainty, reduce it to aniline and test for that. For this purpose vigorously agitate the globules, separated as completely as possible from water, with granulated tin and a few cc. of

[1] A. Rossi: Changes in Nitrobenzene during Cadaveric Putrefaction. Boll. Chim. Farm. 53 (1914), 65.

concentrated hydrochloric acid until there is no odor of nitrobenzene. Pour the acid solution from undissolved tin and add an excess of potassium hydroxide solution to decompose the double chloride of aniline and tin. Extract free aniline with ether. Separate the ether solution and evaporate or distil. Dissolve the residual oil-drops by agitation with water and use this solution for the hypochlorite and phenyl-isocyanide test (see page 72).

ANILINE

Aniline, $C_6H_5.NH_2$, is a colorless, oily, highly refracting liquid having a characteristic aromatic odor and a burning taste. Exposed to air it soon takes on a yellow to brown color and in the end is completely resinified. It boils at 183°. Aniline is only slightly soluble in water (1:31) but mixes in every proportion with alcohol, ether, benzene, chloroform and carbon disulphide. It is without action upon litmus paper. In aqueous solution aniline salts exhibit an acid reaction.

Preparation.—Aniline is prepared by reducing nitrobenzene by means of nascent hydrogen:

$$C_6H_5.NO_2 + 6H = C_6H_5.NH_2 + 2H_2O.$$

Place in a cast-iron pan provided with a stirrer equal quantities by weight of nitrobenzene and water. Then gradually add iron turnings and concentrated hydrochloric acid. In the technical process a smaller quantity of hydrochloric acid is sufficient to bring about complete reduction, since the ferrous chloride first formed acts as a carrier of hydrogen, imparting to the metallic iron the property of reducing nitrobenzene to aniline in presence of water. Aniline is contained in the reaction-mixture partly as the hydrochloride. The latter is decomposed by calcium hydroxide and free aniline is then distilled off by means of steam:

(a) $C_6H_5.NO_2 + 3Fe + 6HCl = C_6H_5.NH_2 + 3FeCl_2 + 2H_2O,$
(b) $C_6H_5.NO_2 + 3Fe + 4H_2O = C_6H_5.NH_2 + 3Fe(OH)_2,$
(c) $C_6H_5.NH_2 + HCl = C_6H_5.NH_2.HCl,$
(d) $2C_6H_5.NH_2.HCl + Ca(OH)_2 = 2C_6H_5.NH_2 + CaCl_2 + 2H_2O.$

Toxic Action.—Aniline is moderately toxic in its action. Doses of 1.5–2 grams, administered in the course of a day, have proved fatal to small dogs. It is not possible to state definitely the average lethal dose for human beings. Very serious results are said to have followed a dose of 3–4 grams of aniline. The lethal dose is certainly less than 25 grams, for that quantity of aniline was sufficient to kill a healthy man. Even inhalation of aniline vapor may cause severe or fatal intoxications.

Aniline produces methaemoglobin and therefore poisons the blood. The conversion of oxyhaemoglobin into methaemoglobin by aniline may be demonstrated by adding an aqueous aniline solution to blood in a test-tube. Aniline

changes their form and partially decomposes red blood-corpuscles. Thereby the quantity of available oxygen in the blood is so diminished that it amounts to only 5–10 volumes instead of 15–20, the normal quantity. The number of red blood-corpuscles is diminished in aniline poisoning but not that of the white blood-cells.

R. v. Engelhardt[1] has shown that aniline is partly changed in the human organism into aniline black, or into a similar compound insoluble in water. At the climax of aniline poisoning blue-black granules may be seen in every drop of blood and also in the urine. The organism rids itself of aniline by oxidizing it to p-amino-phenol, $HO.C_6H_4.NH_2$ (1, 4), which like all phenols combines with sulphuric acid to form an ethereal sulphuric acid, that is, p-amino-phenyl-sulphuric acid, $HO.SO_2.O.C_6H_4.NH_2$ (1, 4). The latter in the form of an alkali salt is eliminated through the kidneys and then appears in the urine. A part of the p-amino-phenol is also eliminated as a conjugate of glycuronic acid. The reduction of Fehling's solution by urine containing aniline is due to this conjugated acid. In severe cases of poisoning, unchanged aniline has also been found in the urine. Usually urine that contains aniline has a very dark color. In addition to the substances mentioned, a dark pigment has been detected in urine in aniline poisoning, as well as haemoglobin, methaemoglobin and an abundance of urobilin.

Detection of Aniline

Aniline is a rather feeble base and part of it will pass over with steam, when a solution acidified with tartaric acid is distilled. To estimate aniline quantitatively in any material, distillation should be as complete as possible. Mix the substance with water, make strongly alkaline with sodium hydroxide or carbonate solution, and distil in a current of steam. Since 30 parts of water at 15° dissolve 1 part of aniline, a considerable amount of this base may remain in solution in the distillate. When the quantity is large, oil-drops will appear. An aqueous aniline solution (aniline water) colors pine wood and elder pith intensely yellow. The following tests for aniline should be applied to the distillate:

1. **Hypochlorite Test.**—Add a few drops of aqueous calcium or sodium hypochlorite solution drop by drop to a portion of the distillate. A violet-blue, or more of a purple-violet color, gradually changing to a dirty red, will appear if aniline is present. Addition of a little dilute aqueous phenol solution containing some ammonia will produce a blue color that is quite stable. This test is sensitive 1:66,000.

2. **Phenyl-isocyanide Test.**—Heat a portion of the distillate with a few drops of chloroform and potassium hydroxide solution. The repulsive odor of phenyl-isocyanide will show the presence of aniline.

[1] Contributions to the Toxicology of Aniline. Franz. Diss. Dorpat 1888.

3. **Bromine Water Test.**—This reagent added to a solution containing aniline will produce a flesh-colored precipitate. This test is sensitive 1:66,000.

4. **Chromic Acid Test.**—Rub in a porcelain dish a trace of pure, absolute aniline with 4–5 drops of concentrated sulphuric acid and add a drop of an aqueous potassium dichromate solution. After a few minutes, the mixture beginning at the edge will take on a pure blue color. Addition of 1–2 drops of water will produce at once a deep blue color. To apply this test to the distillate, first extract with ether, evaporate the ether solution, and test an oily residue as described.

ALCOHOL[1]

Alcohol, C_2H_5OH, brought into contact with many different parts of the organism is very rapidly absorbed, but especially easily from an empty stomach. Although there is practically no absorption of non-volatile aqueous liquids from the stomach, alcohol is freely absorbed. After absorption it passes into the blood and is then distributed to all organs (see chloral hydrate). Experiments upon dogs, colts and adult horses have shown that blood at the climax of narcosis contains 0.72 per cent. of alcohol. There is stupor even when 0.12 per cent. is present. There is difference of opinion among toxicologists with regard to acute alcoholic intoxication, as to whether the poison is uniformly distributed throughout the body, or accumulated in the brain in larger quantity than in other organs. The following percentages of alcohol, found in the organs of a man who had died at the climax of acute alcohol poisoning, lend support to the latter view: liver 0.21, brain 0.47 and blood 0.33 per cent. These figures become intelligible only when it is assumed that alcohol is combined in the brain. But red blood-corpuscles combine with alcohol as they do with other narcotics.

Uncertainty with regard to the subsequent fate of alcohol in the organism has finally been removed as the result of a series of thorough investigations. These experiments have shown that alcohol is never eliminated unchanged through the skin. At most only 1–1.5 per cent. passes off through the kidneys and only 1.6–2 per cent. through the lungs. Strassmann[2] found the quantity eliminated by

[1] In this book the word "alcohol," unqualified by an adjective, such as methyl, amyl, etc., means ethyl alcohol. Tr.

[2] Pflüger's Archiv 49 (1891), 315.

the lungs somewhat higher (5–6 per cent.) and by the kidneys 1–2.5 per cent. The remainder is completely oxidized in the human organism to carbon dioxide and water.

Distribution of Alcohol in Alcohol Poisoning

B. Fischer found the following quantities of alcohol in organs removed from a man who had probably died from drinking too much brandy:

Weight	Organ	Alcohol
2720 grams	Contents of stomach and intestines	30.6 grams
2070 grams	Heart, lungs and blood	10.9 grams
1820 grams	Kidneys and liver	7.8 grams
1365 grams	Brain	4.8 grams

In the internal organs of the cadaver of a man who had died without doubt as the result of acute alcohol poisoning from brandy, A. Juckenack[1] found the following percentages of alcohol: blood from the heart and large blood-vessels 0.53, parts of the heart, lungs, omentum and spleen 0.44, stomach and oesophagus together with contents 0.72, kidneys 0.37, urine 0.65, liver and gall-bladder 0.24, and brain 0.42 per cent.

Detection of Alcohol

Alcohol distils easily with steam and consequently most of it will be in the first fraction. If present in sufficient quantity, it can be recognized in the distillate by its odor. The following tests should be applied to the distillate:

Fig. 12.—Iodoform crystals, from 0.1 gram of alcohol in 5 cc. of a mixture of water, iodo-potassium iodide and potassium hydroxide.

1. Lieben's Iodoform Test.[2]—Gently warm the liquid (40–50°), add a few cc. of aqueous iodo-potassium iodide solution, or a small crystal of iodine, and enough potassium hydroxide solution to give the liquid a distinct yellow to faint brownish color. If alcohol is present, a yellowish white to lemon-yellow precipitate of iodoform will appear immediately, or as the solution cools. If the quantity of alcohol is very small, a precipitate will form upon long standing. When iodoform is deposited slowly, the crystals are very perfect hexagonal plates and stars (Fig. 12).

[1] A. Juckenack: Contribution to the Chapter "Alcohol Poisoning." Zeitschr. f. Unters. d. Nahrgs.-u. Genussm. 16 (1908), 732.

[2] A. Lieben: Formation of Iodoform and Use of this Reaction in Chemical Analysis. Annalen d. Chem. Suppl. 7 (1870), 218.

Notes.—Lieben's iodoform test is very delicate but not characteristic of alcohol. Other primary alcohols, except methyl alcohol, and many secondary alcohols, as well as aldehydes, ketones, acetic ether, aceto-acetic ester, lactic acid, etc., also give iodoform with iodine and potassium hydroxide or alkaline carbonate.

The correct explanation of the iodoform reaction is probably the following: Iodine and potassium hydroxide form potassium hypo-iodite (KOI) by equation (α). This compound brings about the oxidation of alcohol to acetic aldehyde (β) and at the same time substitutes iodine for hydrogen in the latter (γ). Finally tri-iodo-acetic aldehyde is decomposed by the excess of potassium hydroxide into iodoform and potassium formate (δ):

$$(\alpha) \quad 2KOH + I_2 = KI + H_2O + KOI,$$
$$(\beta) \quad CH_3.CH_2.OH + KOI = CH_3.CHO + H_2O + KI,$$
$$(\gamma) \quad CH_3.CHO + 3KOI = 3KOH + CI_3.CHO,$$
$$(\delta) \quad CI_3.CHO + KOH = CHI_3 + H.COOK.$$

2. Berthelot's Test.—Shake the distillate with a few drops of benzoyl chloride, $C_6H_5.CO.Cl$, and an excess of 10 per cent. sodium hydroxide solution until the irritating odor of benzoyl chloride has gone. The aromatic odor of ethyl benzoate will appear. Ten cc. of 0.5 per cent. alcohol will give a distinct odor of this ester:

$$C_6H_5.COCl + C_2H_5.OH + NaOH = C_6H_5.COOC_2H_5 + NaCl + H_2O.$$

3. Chromic Acid Test.—Warm the liquid containing alcohol with dilute sulphuric or hydrochloric acid, and add 1–2 drops of very dilute potassium dichromate solution. The color of the liquid will change from red to green, and at the same time the odor of acetaldehyde will be recognized. This test is not characteristic of alcohol because many other volatile organic compounds behave similarly:

$$3C_2H_5.OH + 4H_2SO_4 + K_2Cr_2O_7 = 3CH_3.CHO + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O$$

4. Ethyl Acetate Test.—Mix the liquid containing alcohol with the same volume of concentrated sulphuric acid. Add a very small quantity of anhydrous sodium acetate and heat. Ethyl acetate will be recognized by its odor:

$$(\alpha) \quad C_2H_5.OH + H_2SO_4 = C_2H_5O.SO_2.OH + H_2O,$$
$$(\beta) \quad CH_3.CO.ONa + C_2H_5O.SO_2.OH = CH_3.CO.OC_2H_5 + NaHSO_4.$$

5. Vitali's Test.—Thoroughly mix a few cc. of distillate in a glass dish with a small piece of solid potassium hydroxide and 2–3 drops of carbon disulphide. Let this mixture stand for a short time without warming. When most of the carbon disulphide has evaporated, add a drop of ammonium molybdate solution (1:10) and then an excess of dilute sulphuric acid. If the distillate contains alcohol,

a red color will appear. Potassium xanthogenate, $SC(OC_2H_5)(SK)$, is first formed. This compound gives a red color with ammonium molybdate. This test is given distinctly by 5 per cent. alcohol. Acetone and acetaldehyde produce a similar color.

ACETONE

Acetone, $(CH_3)_2CO$, is a colorless liquid of characteristic odor, boiling at 56° and miscible in every proportion with water, alcohol, ether and chloroform. Human urine almost always contains a very small quantity of acetone, about 0.01 gram daily, as a physiological constituent. Under pathological conditions, especially in diabetes mellitus (diabetic acetonuria), urine contains much more. It is also present in urine in prolonged high fever, digestive disturbances, severe forms of carcinoma (carcinomatous acetonuria), etc. Finally acetone has been found in urine in considerable quantity in various intoxications (toxic acetonuria), for example, in poisoning by phosphorus, carbon monoxide, atropine, curare, antipyrine, pyrodine, sulphuric acid, extract of male fern; in chronic lead poisoning; and in chronic morphinism after discontinuance of the drug.

Drugs causing an increase in the formation of acetone in man are benzene, salol, benzonaphthol and heroine (diacetyl-morphine) (R. Kobert "Intoxikationen").

Acetone is not poisonous nor in the least corrosive. Man and animals can tolerate considerable quantities of acetone taken internally. It seems to produce no effect, though it may possibly possess very feeble narcotic properties. Archangelsky found that dogs show signs of narcosis when the blood contains 0.5 per cent. of acetone. Even smaller doses produce narcosis in rabbits and have an injurious action upon the blood and kidneys.

Distillates from human urine, as well as from blood and various organs, such as liver, spleen, kidneys, brain, etc., often contain acetone, or more correctly perhaps, substances like acetone. This is especially the case when cadaveric material has begun to putrefy.

Detection of Acetone

Acetone distils easily with steam and therefore is found in the first fraction of the distillate. This should be used for the following tests:

1. Lieben's Iodoform Test.—Add a few cc. of aqueous iodo-potassium iodide solution, or a small crystal of iodine, to an aqueous solution of acetone and then potassium hydroxide solution drop by drop until the color is faint yellow. Iodoform immediately separates, even in the cold, as a yellowish white precipitate which is usually amorphous. Acetone differs from alcohol in giving iodoform, when ammonium hydroxide solution is substituted for potassium or sodium hydroxide solution (Gunning's acetone test). Acetaldehyde resembles acetone in giving iodoform in the cold and under conditions the same as those stated above.

Note.—Potassium hypo-iodite (α) probably converts acetone into tri-iodo-acetone, $CH_3.CO.CI_3$ (β), and this compound is then decomposed by potassium hydroxide into iodoform and potassium acetate (γ):

$$(\alpha) \qquad 6KOH + 3I_2 = 3KI + 3KOI + 3H_2O,$$
$$(\beta) \quad CH_3.CO.CH_3 + 3KOI = CH_3.CO.CI_3 + 3KOH,$$
$$(\gamma) \quad CH_3.CO.CI_3 + KOH = CHI_3 + CH_3.COOK.$$

2. **Legal's Test.**—Add a few drops of freshly prepared sodium nitroprusside solution to a liquid containing acetone, and then potassium hydroxide solution. A red or reddish yellow color will appear. This color soon changes to yellow. Add an excess of acetic acid to the solution. The solution will now have a carmine to purplish red color, according to the quantity of acetone present. Heat will change this color to violet.

This test is given by acetaldehyde but not by alcohol. The red color caused by aldehyde fades upon addition of acetic acid and changes to green with heat. Le Nobel states that ammonium hydroxide, or ammonium carbonate solution, may be substituted for potassium hydroxide solution in Legal's test, but under these conditions the red color is very slow to appear. Le Nobel's modification, however, eliminates the possibility of confusing acetone with acetaldehyde.

3. **Penzoldt's Test.**—Prepare a hot, saturated, aqueous solution of o-nitrobenzaldehyde, $C_6H_4(NO_2)CHO$ (1, 2), and allow it to cool. Add this solution to the liquid containing acetone and also some sodium hydroxide solution. At first the color of the mixture is yellow. It then becomes green and a blue precipitate of indigotine is formed in 10–15 minutes. When indigotine is present in traces only, shake the solution with chloroform. This solvent will dissolve the coloring-matter and become blue.

4. **Reynold's Test.**—Acetone will dissolve freshly precipitated mercuric oxide and this test is based upon this property. Add mercuric chloride solution to the distillate and an alcoholic potassium hydroxide solution. Shake thoroughly and filter. Add ammonium sulphide solution to the clear filtrate as an upper layer. If acetone is present, there will be a black zone (HgS) where the two solutions meet. Use a double filter for filtering the excess of mercuric oxide and continue pouring the filtrate through the paper until it is perfectly clear. According to v. Jaksch, acetaldehyde also dissolves mercuric oxide without reduction which has been confirmed by Salkowski.

Detection of Acetone in Urine

Acidify 200–500 cc. of urine with a few drops of dilute sulphuric acid and distil from a capacious flask. Collect 20–30 cc. of distillate. This will contain almost the entire quantity of acetone in the urine. Acetone thus obtained may possibly be derived from aceto-acetic acid often present in human urine, especially in a severe case of *diabetes mellitus*. Distillation decomposes this acid into acetone and carbon dioxide:

$$CH_3.CO.CH_2.COOH = CH_3.CO.CH_3 + CO_2.$$

Detection of Alcohol and Acetone in Mixtures

Alcohol may be detected in presence of acetone by the benzoyl chloride reaction. On the other hand, acetone may be distinguished from ethyl alcohol by Legal's or Penzoldt's test.

ISOAMYL ALCOHOL

Isoamyl alcohol, usually known as amyl alcohol, has the formula, $(CH_3)_2$.-$CH.CH_2.CH_2.OH$, and is the chief component of the so-called fusel oil. The latter formed together with alcohol during alcoholic fermentation imparts to brandy its unpleasant fusel odor and consists of a mixture of higher alcohols. But fusel oil also contains other substances, for example, furfural. The vapors of amyl alcohol, to which the chemist in his analytical work must occasionally expose himself, are very unpleasant. They may give rise to headache, palpitation of the heart, a feeling of pressure upon the chest, and coughing. Following large doses of amyl alcohol, severe intoxications may appear, manifesting themselves in suspension of respiration, unconsciousness and cyanosis. The presence of alcohol is said to increase its toxicity, for its absorption is made easier. A content of 0.3 per cent. of amyl alcohol in alcohol to be used for drinking purposes should be regarded as inadmissible. A part of the amyl alcohol taken into the system is eliminated as such through the kidneys in the urine.

Amyl alcohol is derived from leucine as a result of yeast fermentation:

$$(CH_3)_2{:}CH.CH_2.CH(NH_2).COOH + H_2O = CO_2 + NH_3 + (CH_3)_2{:}CH.CH_2.CH_2.OH.$$

The formation of fusel oil during alcoholic fermentation is attributed to this reaction.

Detection of Isoamyl Alcohol

More than traces of amyl alcohol can be recognized in material by its specific odor. To detect amyl alcohol with greater certainty, acidify the material and distil with steam. Extract the distillate with chloroform and evaporate the latter at as low a temperature as possible. Amyl alcohol will appear in the form of oil-drops having a characteristic odor. In case the material available is not too small, prepare a saturated solution by shaking a portion of the oil-drops with water at 15–16°. If this solution is warmed to about 50°, it will become milky, provided amyl alcohol is present. Amyl alcohol dissolves at 13.5° in 39 parts of water and at 16.5° in 50 parts. In addition, use the residue from the chloroform solution for the following tests:

1. **Marquardt's Test.**[1]—Put the residue into a test-tube with a little water and add a few drops of dilute sulphuric acid. Then gradually add sufficient potassium permanganate solution (1:1000) to give the mixture a red color after 24 hours. Allow the test-tube to stand corked, adding more permanganate solution, if decolorization takes place within the stated time, and test the odor frequently. If the material contains amyl alcohol, as the tube is opened from time to time there will be detected in succession the odors of i-valerianic aldehyde, i-amylester of i-valerianic acid, and finally after about 24 hours i-valerianic acid. The odor of this acid will appear more marked, if the test-tube is gently warmed in the water-bath. Even when the quantity of fusel oil is very small, this odor is so powerful that it is not masked by many foreign odors.

[1] L. Marquardt: Quantitative Estimation of Fusel Oil in Brandy. Ber. d. Deutsch. chem. Ges. 15 (1882), 1370, 1661.

2. **Uffelmann's Test.**[1]—Add to the oil-drops left by evaporation of the chloroform extract a freshly prepared solution of methyl violet colored green by hydrochloric acid. In presence of i-amyl alcohol the drops are colored violet or more reddish blue.

Detection of Amyl Alcohol in Alcohol and Brandy

Dilute 40 cc. of brandy to 12–15 per cent. with water, that is, to about 500 cc., and then extract with 15 cc. of pure chloroform. Separate the chloroform layer in a separating-funnel and shake it first with the same volume of water. Separate the chloroform from the water and evaporate in a porcelain dish. Use the residue for Marquardt's and Uffelmann's fusel oil tests.

FORMALDEHYDE

Formaldehyde is an aqueous solution containing at least 35 per cent. of CH_2O and known by its officinal name "*Formaldehydum Solutum.*" This solution is also frequently called "*Formalin*" or "*Formol.*" It is a colorless, aqueous liquid having a pungent odor and containing variable quantities of methyl alcohol. Litmus paper is not affected, or at most faintly reddened by it. It is miscible in every proportion with water and alcohol but not with ether.

Physiological Action.—Undiluted formaldehyde solution acts upon animal skin producing a leather-like formation. Even a 3–4 per cent. solution makes the skin rough, hardening the outer layers and gradually killing them. Therefore formaldehyde solutions have the property of hardening animal tissues by coagulating albumin and giving rise to other chemical changes. In this way it prevents them from undergoing putrefaction. These results are sometimes designated as "formalin tanning." Foodstuff, containing protein that has been changed chemically by addition of formaldehyde, partly loses its digestibility and then has less nutritive value. On this account addition of formaldehyde solution to preserve foodstuffs, such as meat, preserved meat, milk, cheese, etc., and also because of the toxicity of formaldehyde is forbidden by law. Even very dilute formaldehyde solutions taken internally bring about hardening, causing changes in the albumin, and killing the cells of the mucous lining of the mouth, stomach and intestines. Officinal undiluted formaldehyde solution has a caustic action and administered internally may produce fatal results in the course of a few hours. The author has examined the stomach and contents as well as parts of the intestines of a man who by mistake drank half a glass (70–80 cc.) of formaldehyde solution, thinking it was fruit-juice. Three hours after the fatal draught he died in the most terrible agony. The urine, blood and internal organs were not submitted for examination. An abundance of formaldehyde was obtained when the material acidified with dilute sulphuric acid was distilled. In this investigation the observation was made that formaldehyde distils very slowly with steam from parts of the cadaver. It was necessary to use a paraffine-bath, to replace the water frequently, and to carry distillation nearly to dryness before even a

[1] Uffelmann: Archiv der Hygiene 1886, 229.

faint test for aldehyde was obtained. Formaldehyde is very resistant toward the putrefactive changes taking place in the cadaver. The author has diluted with water portions of the stomach and intestines, in which formaldehyde had been found, and allowed them to stand for two years in presence of insufficient air. He has then obtained distinct tests for formaldehyde in the distillate from this putrid mass after it had been acidified with dilute sulphuric acid. Even in a rapid current of steam formaldehyde distils very slowly.

Detection of Formaldehyde

1. **General Aldehyde Reactions.**—Formaldehyde exhibits the powerful reducing action of aldehydes, especially upon various metallic oxides. It causes the gradual deposition of metallic silver from silver nitrate solution after addition of ammonia, and decolorizes an alkaline copper tartrate solution (Fehling's solution) upon application of heat with separation of red cuprous oxide. Formaldehyde also produces a gray precipitate of metallic mercury with Nessler's reagent. If, however, a small quantity of very dilute formaldehyde solution is added to Nessler's reagent, a brownish color first appears and then quickly gives place to a gray turbidity. Acetaldehyde gives a brown to gray-brown precipitate with Nessler's reagent.

2. **Reactions with Higher Phenols.**—The particular phenols in these reactions are phloroglucinol and resorcinol. A. v. Baeyer[1] found that formaldehyde forms insoluble condensation-products with univalent and polyvalent phenols when they are warmed in aqueous solution with hydrochloric acid.

(a) **Phloroglucinol Test.**—As a result of a study of the reaction with phloroglucinol, Councler[2] found that it soon gave a turbidity even with very dilute formaldehyde solutions and hydrochloric acid. Gradually this became denser and changed to thick flocks of undetermined composition. This is a very delicate test for formaldehyde.

(α) **Procedure in Acid Solution.**—Gradually heat to boiling the solution to be tested for formaldehyde with a mixture consisting of equal volumes of hydrochloric acid (sp. gr. 1.19 = 37.5 per cent.) and water and sprinkle upon it a little phloroglucinol. After all the phloroglucinol is dissolved, if formaldehyde is present, turbidity and separation of yellow-red flocks appear. In addition to formalde-

[1] A. v. Baeyer: Compounds of Aldehydes with Phenols and Aromatic Hydrocarbons. Ber. d. Deutsch. chem. Ges. 5 (1872), 1094.

[2] C. Councler: Products of the Condensation of Phloroglucinol with Sugars and Aldehydes. Chem.-Ztg. 20 (1896), 585.

hyde, all other compounds containing methylene (CH_2) give this test. But if formaldehyde or other methylene-compounds are not present, this test gives at most a faint yellow color.

(β) **Procedure in Alkaline Solution.**—This test may also be carried out in alkaline solution. Mix 2 cc. of a 0.1 per cent. phloroglucinol solution with 1 cc. of potassium hydroxide solution and add the liquid to be tested for formaldehyde. The appearance of a distinct red color shows presence of formaldehyde. This color test is given only by more dilute formaldehyde solutions, stronger solutions giving no color. A 3 per cent. solution gives only a raspberry-red color, whereas a 0.0004–0.5 per cent. solution changes to a very deep red. To detect formaldehyde in milk, add to 10 cc. 1–2 cc. of a 0.1 per cent. phloroglucinol solution and a few drops of potassium hydroxide solution (L. Vanino[1]).

(b) **Resorcinol Test.**—To detect formaldehyde, Lebbin[2] uses an aqueous solution of 5 per cent. resorcinol and 40–50 per cent. sodium hydroxide solution, mixing equal volumes of this solution and the aqueous solution to be tested, heating to boiling and keeping at that temperature for about half a minute. Even traces of formaldehyde produce a distinct red color.

Notes.—All proteins, albumoses and peptones, interfere with this reaction, for in their presence only relatively large quantities of formaldehyde can be detected by means of alkaline resorcinol solution. In the case of a substance like milk, formaldehyde must first be distilled off and the distillate used for the test. Urine should first be freed from color by means of bone-black and the resorcinol test for formaldehyde then made with the decolorized urine. Another fact to be borne in mind is that alkaline resorcinol solution produces a color like that of the formaldehyde test with chloroform and those compounds, such as chloral hydrate, that give chloroform with caustic alkalies. The limit of delicacy of the chloroform test with alkaline resorcinol solution is 1:5000 but 1:10,000,000 in case of the formaldehyde test. Therefore in making the resorcinol test the liquid under examination should be considerably diluted when chloroform, or substances that yield chloroform with caustic alkalies, are present.

3. Hehner's Test[3] Modified by Leonard.[4]—Even in high dilution formaldehyde produces a blue-violet color when brought in contact with protein and hydrochloric or sulphuric acid containing ferric chloride.

[1] L. Vanino: Detection of Formaldehyde by Means of Phloroglucinol. Pharm. Zentralbl. 40 (1899), 101.

[2] Lebbin: Detection of Formaldehyde. Pharm. Ztg. 42 (1897), 18.

[3] O. Hehner: Detection of Formalin. The Analyst 21 (1896), 94.

[4] N. Leonard: Hehner's Formaldehyde Test. The Analyst 21 (1896), 157.

Procedure.—Mix in a large test-tube 5 cc. of the liquid to be tested for formaldehyde with 2 cc. of fresh unboiled milk and 7 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.), containing in 100 cc. 0.2 cc. of 10 per cent. ferric chloride solution, and boil gently for about 1 minute. In presence of formaldehyde a violet color will appear.

Notes.—In making this test it must first be established experimentally that the milk used is free from formaldehyde and that it gives the test with a trace of formaldehyde. According to Juckenack, Hehner's test for formaldehyde is sharper than that with morphine-sulphuric acid. Its sensitiveness, however, is strongly influenced when acetaldehyde is present at the same time, especially if the quantity of this aldehyde is quite large. Hehner's test may also be carried out in the manner of a zone-test by carefully adding to pure concentrated sulphuric acid as an upper layer a mixture of 5 cc. of the aqueous liquid to be tested with 5 cc. of fresh milk and a few drops of very dilute ferric chloride solution. If formaldehyde is present, a violet-blue zone will at once appear at the line of contact of the two layers and remain distinctly visible for 2–3 days. In examining milk for formaldehyde by Hehner's test, dilute it with an equal volume of water and add this mixture as an upper layer to concentrated sulphuric acid, containing a trace of ferric chloride. Milk free from formalin gives a greenish color but a violet-blue ring when it is present.

According to E. Salkowski,[1] any albumose, particularly Witte's peptone, may be substituted for milk in Hehner's test with equally good results. Dissolve by shaking and warming a little Witte's peptone in 8 cc. of the liquid to be tested. Then add 3–4 drops of 3 per cent. ferric chloride solution, about half the volume of hydrochloric acid (sp. gr. 1. 19) and heat to boiling. In presence of formaldehyde, a gradually increasing full violet color at once appears and later passes into more of a blue. Hydrogen peroxide may also be substituted for ferric chloride but in excess it destroys the coloring-matter.

Chemical Explanation of Hehner's Formaldehyde Test with Milk

Pure formaldehyde and pure concentrated sulphuric or hydrochloric acid give no color with proteins. For the development of the color-reaction a small quantity of an oxidizing agent is required. In presence of a trace of ferric chloride, potassium nitrite, platinic chloride, sodium peroxide, hydrogen peroxide, sodium percarbonate, ammonium and potassium persulphate, the purple-violet color at once appears. This formaldehyde reaction is characteristic of proteins and is dependent upon the presence of a tryptophane group in the protein molecule. Its intensity varies with different proteins in accordance with the amount of tryptophane they contain. For this reason gelatine does not give this reaction. The certainty with which colors appear in presence of tryptophane, indole and skatole, renders solutions of these substances suitable for the colorimetric estimation of small quantities of formaldehyde.

The test for formaldehyde in milk first described by Hehner is not a general aldehyde reaction but apparently is confined to formaldehyde alone. Moreover

[1] E. Salkowski: Contribution to the Knowledge of Some Formaldehyde Reactions. Biochem. Zeitschr. 68 (1914), 337.

most aromatic hydrocarbons and acids as well as phenols and amino-compounds give no similar color.

To detect formaldehyde in milk, the latter is diluted ten times. This test is more delicate, if half of a mixture of 50 cc. of milk and 20 per cent. phosphoric acid is distilled off. According to v. Fillinger[1] even 0.004 per cent. of formaldehyde can be detected direct in milk but 0.0008 per cent. in the distillate. To detect formaldehyde in butter and meat, melt the former in presence of water under a reflux and then test the aqueous solution; mix the meat with the same weight of 20 per cent. phosphoric acid and then distil off half the total weight. In this way even 0.024 per cent. of formaldehyde can be detected in meat with certainty; with 0.016 per cent. the reaction was considerably weaker.

4. Jorissen's[2] Morphine-Sulphuric Acid Test.—With a solution of morphine hydrochloride in concentrated sulphuric acid, formaldehyde gives a red-violet to blue-violet color that increases in intensity with time, is quite stable and finally passes into indigo-blue.

Procedure.—Before making the test dissolve 0.05 gram of morphine hydrochloride in 2.5 cc. of concentrated sulphuric acid. Also carefully mix with cooling 1 cc. of the aqueous liquid to be tested with 5 cc. of concentrated sulphuric acid. Cool to room temperature and add the morphine-sulphuric acid solution previously prepared. If formaldehyde is present, the mixture will at once take on a purple-red color.

This test may also be made by dissolving in a porcelain dish a crystal of morphine hydrochloride in about 10 drops of dilute sulphuric acid (1 part of concentrated acid and 6 parts of water). Add to this colorless solution by means of a glass rod a drop of the liquid to be tested. Wirthle makes this test by cooling well and mixing 1 cc. of the aqueous liquid with 5 cc. of concentrated sulphuric acid. He does not use, however, a solution of morphine hydrochloride in 2.5 cc. of sulphuric acid but 0.05 gram of the morphine salt itself.

G. Fendler and C. Mannich[3] have compared this test with other formaldehyde tests with regard to sensitiveness. After half a minute a 0.1 per cent. formaldehyde solution had a distinct violet color, after 3 minutes a deep dark-violet; in the course of 10 minutes a 0.01 per cent. solution was distinctly violet, the color increasing with time; within 15 minutes a 0.005 per cent. formaldehyde solution was distinctly violet; within 1 hour a 0.001 per cent. solution had a barely per-

[1] F. v. Fillinger: Detection of Formaldehyde. Zeitschr. f. Unters. d. Nahrgs.- u. Genussm. 16 (1908), 226.

[2] A. Jorissen: Detection of Formalin in Foods. Rev. intern. falsific. 11 (1898), 12.

[3] G. Fendler and C. Mannich: Detection of Methyl Alcohol in Alcoholic Preparations. Arb. a. d. Pharm. Institut der Universität Berlin 3 (1906), 243.

ceptible violet color. Therefore even 0.01 milligram of formaldehyde can be detected in a dilution of 1:100,000. The resorcinol test fails even at a dilution of 1:20,000, whereas the phloroglucinol test is equal to the morphine test in delicacy. The latter, however, has the advantage of being characteristic and can also be observed more easily than the very transient phloroglucinol test. Fendler and Mannich consider the morphine-sulphuric acid test as the most serviceable reaction for the detection of formaldehyde. With this morphine-reagent acetaldehyde gives only yellow colors.

5. Fuchsine-Sulphurous Acid Test. (a) **According to Denigès.**[1]—The color given by fuchsine-sulphurous acid with an equal volume of a sufficiently dilute acetaldehyde solution disappears after a few hours, whereas the color produced by this reagent with formaldehyde increases in intensity with time and besides is very stable. This difference between these two aldehydes manifests itself particularly in strong sulphuric acid solution and serves for the detection of traces of formaldehyde in presence of considerable quantities of acetaldehyde. If, for example, 1.2 cc. of sulphuric acid (sp. gr. 1.66 = 73.64 per cent.) are added for every 5 cc. of an aqueous acetaldehyde solution and fuchsine-sulphurous acid, hardly any color appears after vigorous shaking and prolonged action. But if the acetaldehyde is replaced by a formaldehyde solution, even a trace of the latter produces a blue or more of a blue-violet color. After 5–10 minutes standing, 0.01 milligram of formaldehyde and less can be detected with certainty by a violet color and an absorption-band in the orange. Obviously all formaldehyde derivatives, which like hexamethylene-tetramine (urotropine) yield formaldehyde under the influence of sulphuric acid, as well as all those compounds that like methyl alcohol give formaldehyde when properly treated, respond to this test.

Preparation of Fuchsine-Sulphurous Acid.—Mix a solution of 1 gram of fuchsine in 1 liter of water with 50 cc. of saturated sodium bisulphite solution and acidify with 1 cc. of concentrated sulphuric acid.

(b) **According to Grosse-Bohle-Fincke.**[2]—This formaldehyde reaction differs from the general aldehyde tests with fuchsine-sulphurous acid in having present considerable free hydrochloric acid. Of all

[1] G. Denigès: Detection of Traces of Formaldehyde in Presence of Acetaldehyde by Means of Fuchsine-Bisulphite. Compt. rend. de l'Académie des sciences 150 (1910), 529.

[2] H. Fincke: Detection of Small Quantities of Formaldehyde and of Some Formaldehyde Compounds by Means of Fuchsine-Sulphurous-Hydrochloric Acid. Zeitschr. f. Unters. d. Nahrgs.-u. Genussm. 27 (1914), 246.

acids, hydrochloric acid is best adapted for this purpose, for this reaction is characteristic of formaldehyde at higher acid concentration. Under these conditions other aldehydes do not interfere with this formaldehyde test, unless they are present in too large quantity. Other substances of the most varied character are without influence.

Procedure.—Mix 10 cc. of the liquid to be tested for formaldehyde with 1–2 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.) and then add 1 cc. of the reagent. According to the amount of formaldehyde present, a permanent blue-violet to red-violet color appears within a few minutes or at latest 12 hours. Obviously other substances such as hexamethylene-tetramine and methylal, that undergo decomposition in acid solution giving formaldehyde, also give this test. A successful result is dependent upon the exact observance of instructions.

Preparation of the Reagent.—Dissolve 1 gram of rosaniline acetate or hydrochloride in about 500 cc. of water, add an aqueous solution of 25 grams of crystallized sodium sulphite together with 15 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.), and bring to 1 liter with water.

Detection of Formalin in Milk

(Grosse-Bohle[1])

Add 2 cc. of concentrated hydrochloric acid to 10 cc. of water, shake and add 1 cc. of a fuchsine solution decolorized by means of sulphurous acid, containing 1 gram of pure rosaniline hydrochloride in a liter. Larger amounts of formalin can be recognized after 10–15 minutes, smaller amounts after a few hours, by a red-violet color in the milk. After 12 hours the intensity of color reaches a maximum and may be used for a colorimetric estimation. By this test even 0.005–0.01 cc. of "*Formalin*" in 1 liter, or 1 drop in 5–10 liters of milk, may be detected.

6. Rimini's[2] Phenyl-hydrazine Test.—Add 1 cc. of a dilute aqueous solution of phenyl-hydrazine hydrochloride together with a few drops of a freshly prepared solution of sodium nitro-prusside, and finally a few drops of concentrated sodium hydroxide solution to 10–15 cc. of a very dilute formaldehyde solution. A blue color will appear and later pass into red. This blue color can be recognized in milk even in presence of 1/30,000 part of formaldehyde.

[1] H. Grosse-Bohle: The Hygienic Inspection of the Milk Business. Zeitschr. f. Unters. d. Nahrgs.-u. Genussm. 14 (1907), 78.

[2] E. Rimini: Detection of Formaldehyde in Foods. Ann. di Farm. 3 (1898), 97; Chem. Zentralbl. 1898, I, 1152.

In this reaction ferric chloride in hydrochloric acid solution may be substituted for sodium nitro-prusside in alkaline solution in the following manner. Add in succession to 10 cc. of very dilute formaldehyde solution 2 cc. of a 4 per cent. aqueous solution of phenyl-hydrazine hydrochloride, 6 drops of 10 per cent. ferric chloride solution, and 5 cc. of hydrochloric acid. This mixture will have a raspberry-red color. Under the same conditions acetaldehyde gives a light yellow color. These two formaldehyde tests are delicate and according to E. Rimini are given by no other aldehyde. They are adapted for the detection of formaldehyde in foods, especially in milk.

According to Meth,[1] the test last described is also given by acrolein but the shade of color is somewhat different and it is less sensitive. In examining fats that have become rancid, to avoid error, Meth employs the very sensitive first test of Rimini with phenyl-hydrazine hydrochloride, sodium nitro-prusside and sodium hydroxide solution. In this case the blue color is produced only by formaldehyde and not by acrolein.

Schryver's[2] Procedure

Add 2 cc. of a freshly prepared and filtered 1 per cent. solution of phenyl-hydrazine hydrochloride, then 1 cc. of a fresh 5 per cent. potassium ferricyanide solution, and finally 5 cc. of concentrated hydrochloric acid to 10 cc. of an aqueous solution containing free formaldehyde. A splendid color similar to fuchsine will appear. In the case of combined formaldehyde, for example, hexa-methylene-tetramine, warm the mixture for a short time after adding phenyl-hydrazine hydrochloride or allow it to stand, and then add potassium ferricyanide and hydrochloric acid. In presence of other dyestuffs, especially when only small amounts of formaldehyde are present, dilute with water after adding the reagents. Then add ether and shake. The hydrochloride of the chromatogenic base undergoes dissociation and the latter is dissolved by the ether. Shake the ether solution with a little concentrated hydrochloric acid which will take up the dyestuff as the colored hydrochloride. Acetaldehyde does not give this test. Furfural gives with this mixture an apricot-yellow color, which becomes dirty green with concentrated hydrochloric acid, is transient, and is not given at all in a concentration of 1:100,000. This test was employed for the detection of formaldehyde synthesized through the agency of chlorophyll.

7. Hexamethylene-tetramine Test.—Formaldehyde easily condenses with ammonia forming hexamethylene-tetramine:

$$6CH_2O + 4NH_3 = 6H_2O + (CH_2)_6N_4.$$

With mercuric chloride and potassium mercuric iodide it gives characteristic crystalline precipitates. Add ammonia repeatedly and evaporate to dryness in a porcelain dish upon the water-bath the liquid to be tested for formaldehyde, for example, the distillate

[1] Meth: Detection of Formaldehyde. Chem.-Ztg. 30 (1906), 666.

[2] S. B. Schryver: The Photo-Chemical Formation of Formaldehyde in Green Plants. Proc. Roy. Soc. London, Series 82 (1910), 226.

obtained from any kind of material. If more than traces of formaldehyde are present, the residue will contain the characteristic crystals of hexamethylene-tetramine. They may be recognized by dissolving them in 4–5 drops of water, placing a drop of the solution upon a glass slide, and examining under the microscope.

(a) Add to one drop a drop of saturated mercuric chloride solution. If hexamethylene-tetramine is present, a crystalline precipitate will immediately appear. In a short time star-shaped crystals having three and more rays will be seen and later perfect octahedrons.

(b) Add to a second drop a drop of potassium mercuric iodide solution and very little dilute hydrochloric acid. If hexamethylene-tetramine is present, light yellow hexagonal stars will form.

8. Codeine and Sulphuric Acid Test.—Dissolve codeine in a little concentrated sulphuric acid and then add a drop of the liquid to be tested for formaldehyde. An intense blue color will appear if formaldehyde is present. Acetaldehyde and furfural do not give this blue color. An absolutely pure sulphuric acid free from ferric oxide must be used for this test, otherwise an impure acid of that description will itself produce a blue color with codeine. Dilute sulphuric acid may also be substituted for the concentrated acid.

Quantitative Estimation of Formaldehyde

Various volumetric methods have been devised for the quantitative estimation of formaldehyde in aqueous solution. Two of these will be described here. Other methods of estimating formaldehyde will be found in Chapter V on pages 548 to 551.

1. Romijn's[1] Iodimetric Method

This method is based upon the easy oxidation of formaldehyde to formic acid by iodine in alkaline solution:

$$H.CHO + H_2O + I_2 + 3NaOH = H.COONa + 2NaI + 3H_2O.$$

Oxidation is complete within 10 minutes. Iodine must always be present in excess. That not combined with formaldehyde will react with sodium hydroxide:

$$2NaOH + I_2 = NaI + NaOI + H_2O.$$

If the alkaline solution is acidified, iodine will again be set free:

$$NaI + NaOI + 2HCl = I_2 + 2NaCl + H_2O.$$

Finally iodine thus liberated is estimated by titration with 0.1 n-sodium thiosulphate solution:

$$2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6.$$

[1] G. Romijn: Estimation of Formaldehyde. Zeitschr. f. analyt. Chem. 36 (1897), 18.

Procedure.—Mix in a glass-stoppered flask 10 cc. of a formaldehyde solution, diluted to a definite volume, with 25 cc. of 0.1 n-iodine solution and sufficient strong sodium hydroxide solution to produce a light yellow color. Stopper the flask and allow the mixture to stand for 10 minutes protected from light. Then add dilute hydrochloric acid until the color is deep brown and titrate free iodine with 0.1 n-sodium thiosulphate solution, using starch solution as indicator.

Calculation.—According to the equation, 1 molecule of formaldehyde requires 2 atoms of iodine; 1000 cc. of 0.1 n-iodine solution, containing 0.1 gram-atomic weight of iodine, correspond therefore to $\frac{CH_2O}{20}$ grams $= \frac{30.02}{20} = 1.501$ grams of formaldehyde and 1 cc. of 0.1 n-iodine solution corresponds to 0.001501 gram of CH_2O.

Example.—10 cc. of very dilute formaldehyde solution were taken. 25 cc. of 0.1 n-iodine solution were used. By titration 10.2 cc. of 0.1 n-iodine remained uncombined. Therefore the formaldehyde required $25 - 10.2 = 14.8$ cc. of 0.1 n-iodine. This volume corresponds to $14.8 \times 0.001501 = 0.0223$ gram of CH_2O contained in the 10 cc. of formaldehyde solution taken.

Notes.—Romijn's method is very useful when pure formaldehyde solutions are used. But it is not applicable to solutions that contain other aldehydes, alcohol, acetone, or any substances that will combine with iodine.

2. Sodium Sulphite Method

(Lemme[1] and Doby[2])

Formaldehyde combines very easily with sodium bisulphite to form oxy-methyl-sodium sulphonate:

$$H_2CO + NaHSO_3 = H_2C\begin{matrix}OH\\ \diagdown\\ \diagup\\ SO_3Na\end{matrix}$$

This tendency of formaldehyde to form its bisulphite compound is so strong that even in a neutral solution of sodium sulphite, Na_2SO_3, the reaction takes place at once and at the same time a corresponding equivalent of sodium hydroxide is set free (G. Lemme):

$$H_2CO + Na_2SO_3 + H_2O = H_2C\begin{matrix}OH\\ \diagdown\\ \diagup\\ SO_3Na\end{matrix} + NaOH.$$

[1] G. Lemme: Estimation of Formaldehyde in Solutions. Chem. Ztg. 27 (1903), 896.

[2] G. Doby: Estimation of Commercial Formaldehyde. Zeitschr. f. angew. Chem. 20 (1907), 353.

This free sodium hydroxide is then estimated volumetrically and in that way the amount of formaldehyde in a pure or crude solution can be determined. Since aqueous solutions of sodium sulphite react alkaline to phenol-phthalein, the red color produced by this indicator must first be discharged by addition of a few drops of sodium bisulphite solution. Doby recommends rosolic acid as an indicator, since its behavior toward sodium sulphite solution is entirely neutral.

Requirements. 1. 2 n-Sodium Sulphite Solution.—Dissolve 252.3 grams of crystallized sodium sulphite, $Na_2SO_3.7H_2O$, in water to 1 liter. This solution does not keep indefinitely, since sulphites owing to external catalytic influences go over into sulphates in presence of air. Therefore the quantity of sodium sulphite in solutions that have been kept for some time should be determined beforehand by titration with 0.1 n-iodine solution.

2. Rosolic Acid Solution.—Dissolve 1 gram of rosolic acid in 40 cc. of 50 per cent. alcohol.

Procedure.—If formaldehyde is to be estimated in a commercial formalin solution containing 35–40 per cent., use a solution that has been diluted ten times. According to Doby, 25 cc. of formalin are weighed in a 100 cc. flask and water is added to the mark. Add to 10 cc. of this diluted solution 50 cc. of sodium sulphite solution, previously neutralized with normal acid, and 2 drops of rosolic acid solution. Titrate the solution that is now red with n-hydrochloric acid. According to the above equation, 1 molecule of sodium hydroxide is set free for 1 molecule of formaldehyde. Therefore 1000 cc. of n-acid correspond to a gram-molecular weight = CH_2O grams = 30 grams of formaldehyde and 1 cc. of n-acid corresponds to 0.03 gram of CH_2O.

Notes.—This method is especially well adapted for the estimation of formaldehyde in aqueous solutions containing iron such as collect in the formalin vacuum apparatus. Dissolved iron, which would interfere with the procedure, is converted into a complex compound by addition of neutral Rochelle salt so that no precipitation of iron in the alkaline liquid takes place. If, however, the formaldehyde solution is already turbid owing to separation of iron, phenol-phthalein is to be preferred to rosolic acid as indicator, because it imparts a stronger red color to the alkaline liquid.

METHYL ALCOHOL

Methyl alcohol or wood spirit, $CH_3.OH$, like ethyl alcohol is a colorless liquid of characteristic odor, boiling between 66.2–66.8° and miscible in every proportion with alcohol, acetone, ether and

chloroform. Methyl alcohol is an excellent solvent for fats, oils, resins and varnishes and is frequently used instead of alcohol for this purpose.

Physiological Action.—Methyl alcohol like alcohol is intoxicating. Taken in larger quantities, especially in concentrated form, it is toxic in its action. In its capacity as a solvent of fats methyl alcohol should exert a very strong haemolytic action. This, however, is not the case, at least its haemolytic action is not as strong as that of alcohol. But it acts as a local irritant poison upon those mucous surfaces with which it comes in contact. Owing to its solvent action upon lipoids it exerts its toxic power by paralyzing the central nervous system. Finally the gross anatomical changes that it causes in various organs take place more rapidly and are more severe than those produced by alcohol. In more serious poisoning by methyl alcohol, when death does not ensue, frequently dangerous derangement of vision results which may end in total blindness. These disturbances may appear in a few hours even after taking a dose of methyl alcohol that is not lethal. Very frequently cystitis is an after-effect of methyl alcohol poisoning, a possible explanation of which may be that the poison leaves the body in a form irritating to the urinary passage. Methyl alcohol, at least in part, is oxidized to formic acid in the animal organism. According to experiments made by Autenrieth,[1] a man, who in a period of 8 days had imbibed 80 grams of pure methyl alcohol in a very dilute form, excreted during this time 5.6 grams more formic acid than he had for the same time under normal conditions. A remarkable fact in this case was that the maximum amount of formic acid was not found in the urine until the third and fourth day after the last dose of methyl alcohol had been taken. Since formic acid is a normal constituent of human urine, obviously a mere qualitative test for its presence does not afford sufficient grounds for coming to a definite conclusion with regard to suspected methyl alcohol poisoning. In such cases a quantitative estimation of formic acid in the urine of the person supposed to have been poisoned by methyl alcohol is absolutely essential. If the formic acid in the urine shows a marked increase, even after entirely harmless quantities of methyl alcohol have been taken, this will be all the more the case in severer poisoning, especially when it terminates fatally. After internal administration of methyl alcohol, formaldehyde can never be found in the urine, but a trace of unchanged alcohol may be detected, that is, when 30 grams of pure methyl alcohol in very dilute form have been taken in the course of 2 days. Methyl alcohol is retained in the human body for some time and is only slowly oxidized. This fact is proved by the results of Autenrieth's experiments which show that the maximum quantity of formic acid is not eliminated until the third and fourth day after the last dose of methyl alcohol. For this reason methyl alcohol can still be detected in the cadaver even when death does not ensue for a day after the last drink of this alcohol. The quantity of formic acid in the urine of the person poisoned will also show a marked increase. In methyl alcohol poisoning the liver is usually enlarged and very fatty. In the

[1] W. Autenrieth: Amount of Formic Acid Normally Present in Human Urine and after Administration of Different Substances. Münch. med. Wochenschr. 1919, No. 31; page 862.

case of the dog, Pohl[1] found that 16.6 per cent. was the average normal amount of fat in the liver, whereas in the case of an animal poisoned by methyl alcohol it rose to 37.9 per cent. Following methyl alcohol poisoning, the spleen in man is usually found enlarged.

Detection of Methyl Alcohol

Methyl alcohol differs from alcohol in not forming iodoform with iodine and solutions of alkaline hydroxides or carbonates. Most of the methods for its detection depend upon two different reactions. Either methyl alcohol is oxidized to formaldehyde and this is detected; or it is converted by means of iodine and red phosphorus into methyl iodide, the latter into dimethyl-aniline, and this compound into methyl violet by means of an oxidizing agent. The analogous diethylaniline from alcohol does not give this result. In many instances methyl alcohol has to be detected in presence of alcohol. In such a case the positive detection of methyl alcohol, against which no objection can be raised, is more or less difficult. In toxicological-chemical investigations the alcohol usually has to be distilled off from gastric and intestinal contents, urine, blood or organs. It must be borne in mind in carrying out this operation that methyl alcohol does not distil as easily with steam as might be supposed from its lower boiling-point. Such investigations usually require the detection of small quantities of methyl alcohol in very dilute aqueous solutions, that is, in distillates. Those methods of detection that depend upon the conversion of methyl alcohol into methyl iodide and methyl violet are not applicable for this purpose, although they are very serviceable in the case of pure alcoholic mixtures, for example, in the detection of methyl alcohol in brandies. Those tests too that have been recommended at various times for the direct detection of methyl alcohol in presence of alcohol are also out of the question.

1. **Oxidation Tests for Methyl Alcohol.**—The oxidation of methyl alcohol to formaldehyde may be brought about in a variety of ways. It may be effected by means of a copper spiral superficially oxidized by having been previously brought to a red heat, by potassium dichromate and sulphuric acid, by potassium permanganate, also in presence of sulphuric acid as well as catalytically. In the absence of alcohol, which may be shown by first making the iodoform test, the detection of methyl alcohol is simpler. Obviously these tests,

[1] J. Pohl: Arch. f. exper. Path. u. Pharm. 31 (1895), 286.

when they are positive, only prove the presence of methyl alcohol if formaldehyde as such is absent. Consequently the material should always first be tested for formaldehyde.

(a) **With Glowing Copper Spiral.**—Put the liquid to be tested, for example, distillate, in a test-tube. Then introduce a copper spiral heated to bright redness clear to the bottom of the tube for a very short time, about a second. Repeat this operation 5–6 times, that is, until the lower part of the spiral remains coated with copper oxide. Then test the resulting liquid for formaldehyde by means of one of the color-reactions (see page 80). If this aldehyde is present, the liquid will have more or less of a red color with alkaline phloroglucinol solution. Or make Hehner's test in the following manner described by Kahn.[1] Mix 5–10 cc. of the oxidized liquid with 2–5 cc. of fresh milk and a drop of dilute ferric chloride solution. Carefully add this mixture as an upper layer to a few cc. of concentrated sulphuric acid. In presence of formaldehyde, a violet-blue color will appear within 3 minutes at the zone of contact of the two liquids. The test with morphine-sulphuric acid may also be made. In the copper spiral test, undiluted ethyl alcohol must previously be diluted with water ten times.

(b) **With Potassium Permanganate.**—This reagent oxidizes methyl alcohol to formaldehyde and alcohol to acetic acid.

(α) **Oxidation According to Juckenack.**—Add to 5–10 cc. of the liquid to be tested for methyl alcohol, for example, the distillate from any material, an equal volume of dilute sulphuric acid and a very small quantity of finely ground potassium permanganate. If a reaction does not take place at once, set the mixture in water at 50°. As soon as the red color has gone, add a little more potassium permanganate, keeping the temperature at about 50°. Proceed in this manner until the red color holds for at least 5 minutes. Then allow the solution to stand in warm water at 50° until the color is gone. Finally filter through a small paper, returning the filtrate to the paper until it is clear and colorless. Potassium permanganate that may be present is reduced by the paper and in this way the solution loses its color. The author does not recommend decolorizing the still red liquid by adding a few drops of dilute alcohol. Test a portion of the colorless filtrate for formaldehyde with milk and hydrochloric acid containing ferric chloride and a second portion with

[1] J. Kahn: Detection of Methyl Alcohol in Liquids Containing Alcohol. Pharm. Ztg. 50 (1905), 655.

morphine-sulphuric acid. Make this second test by adding 5 cc. of concentrated sulphuric acid with cooling to 1 cc. of the filtrate and then adding to this cold mixture a freshly prepared solution of 0.05 gram of morphine hydrochloride in 2.5 cc. of concentrated sulphuric acid. The morphine salt itself (0.05 gram) may be added without being first dissolved (Wirthle). The former test is sharper than that with morphine-sulphuric acid but the presence of considerable quantities of acetaldehyde at the same time causes serious interference.

(β) **Oxidation and Denigès' Test.**—According to Denigès, in testing with fuchsine-sulphurous acid for formaldehyde formed by the oxidation of methyl alcohol, the presence of alcohol not only does not interfere but even has a favorable influence, since the formol-acetal, $CH_2(OC_2H_5)_2$, appearing as an intermediate product, reacts especially easily with fuchsine-sulphurous acid. Add to the aqueous liquid to be tested for methyl alcohol (about 6 cc.), which should not contain more than 3–4 per cent. of methyl alcohol, 0.2 cc. of purest alcohol, 5 cc. of 2.5 per cent. potassium permanganate solution, and 0.4 cc. of sulphuric acid. After 2–3 minutes discharge the color with cold saturated oxalic acid solution (about 8 per cent.), adding to the mixture, after it has become madeira-yellow, 1 cc. of sulphuric acid and shaking. As soon as the liquid is colorless, add 5 cc. of fuchsine-sulphurous acid. A violet color will appear at once and, the more methyl alcohol present, the more intense it will be. This color attains its maximum after about an hour. It is still very strong, if 1 per cent of methyl alcohol is present, and visible with 0.1 per cent.

Fendler and Mannich[1] have observed that formaldehyde alone is completely destroyed in a short time by excess of potassium permanganate in acid solution, but that a detectable quantity of formalin remains if an alcoholic solution of formaldehyde is oxidized by an amount of potassium permanganate insufficient for the complete oxidation of the alcohol.

The oxidation of methyl alcohol to formaldehyde by platinum black according to Peratoner and Tamburello and by sodium persulphate according to Pazienti, is given in Autenrieth's "*Chemical Detection and Estimation of Poisons*" which forms a part of Abderhalden's "*Biological Laboratory Methods.*"

[1] G. Fendler and C. Mannich: Detection of Methyl Alcohol in Alcoholic Preparations. Arb. a. d. Pharm. Inst. d. Univers. Berlin 3 (1906), 243.

Detection of Methyl Alcohol in Parts of Cadavers

(Juckenack[1])

Add to the comminuted cadaveric material 15–20 per cent. of sodium chloride, acidify with phosphoric acid, and distil from an oil-bath, the temperature of which is allowed to rise slowly to about 150°, until the weight of the distillate is about half that of the cadaveric material taken. Test the distillate for formaldehyde with milk and ferric chloride-hydrochloric acid according to the procedure described in the "Methods of Procedure of the Meat Inspection Law" (see page 546). If this test is negative, filter from insoluble fatty acids, exactly neutralize the filtrate with sodium hydroxide solution, add 20 per cent. sodium chloride, and distil to about one-third. The residue in the distillation flask may be tested as described for formic acid, whereas the distillate may be examined for methyl alcohol (see page 91). One portion of the colorless filtrate finally obtained after oxidation may be tested for formaldehyde with milk and ferric chloride-hydrochloric acid and a second portion with morphine-sulphuric acid. The former reaction is sharper but considerable quantities of acetaldehyde cause interference.

Formic Acid.—Strongly acidify the neutral residue remaining in the distillation flask with phosphoric acid and then distil off about one-third. A reaction given by mercuric chloride with this distillate should not be accepted under all conditions as conclusive proof of the presence of formic acid, because a distillate from animal organs may contain substances that resemble formic acid in their reducing action. The detection of formic acid in these distillates, as carried out by Juckenack, is based upon an observation of Fenton that formic acid in aqueous solution in presence of sulphuric acid is reduced to formaldehyde by magnesium and can be detected by various tests. With this object in view, render the distillate faintly acid with dilute sulphuric acid and, by adding each time magnesium turnings in small amount, keep up an evolution of hydrogen that is not too violent but is always active. When traces of formic acid (about 0.1 per cent.) are present, this action must be maintained for about an hour, whereas larger quantities require only 2–3 minutes. Finally test the filtrate for formaldehyde with milk and ferric chloride-hydrochloric acid.

Notes.—By the method described, Juckenack was able on the occasion of the poisoning epidemic in Berlin asylums (December 1911) to detect methyl alcohol in quite a large number of different parts of many cadavers, whereas the result was negative in the case of putrefying meat, human faeces, and fresh as well as putrid liver. The fresh liver came from a man who had died as the result of an accident. The liver that had passed into putrefaction was also a human liver and was tested as a control. By conducting distillation from an oil-bath in the manner described, the troublesome frothing that occurs in steam distillations as well as over-heating of material was avoided. In the putrefaction of liver sometimes not inconsiderable quantities of ethyl alcohol are produced as a result of the fermentation of glycogen that has undergone cleavage on account of the presence of the blood-ferments.

[1] A. Juckenack and Collaborators: The Berlin Methyl Alcohol Poisonings; Interesting Observations and Experiences from the Practice of the Food-Chemist. Zeitschr. f. Unters. d. Nahrgs.-u. Genussm. 24 (1912), 7.

2. Autenrieth's[1] p-Bromo-benzoylchloride Test.—In dilute aqueous solution in presence of sodium hydroxide solution, methyl alcohol gives with p-bromo-benzoylchloride the well-crystallized methyl ester of p-bromo-benzoic acid:

$CH_3.OH + C_6H_4Br.CO.Cl + NaOH = C_6H_4Br.COOCH_3 + H_2O + NaCl.$

On the other hand, alcohol under the same conditions gives the corresponding ethyl ester which remains liquid even at low temperatures. Since p-bromo-benzoylchloride melts at 43°, the liquid to be tested for methyl alcohol should first be heated about to this temperature before the reagent is added.

Procedure.—Add to the aqueous solution to be examined, for example, 40–50 cc. of distillate, about 10 cc. of 10 per cent. sodium hydroxide solution, warm the mixture, best in a glass-stoppered flask, to 40–45°, add according to the quantity of methyl alcohol suspected 1–5 grams of ground p-bromo-benzoylchloride, and shake vigorously until cold. The mixture should remain alkaline. From time to time it should be tested with litmus paper. If the reaction becomes acid, further addition of sodium hydroxide solution is necessary. In presence of methyl alcohol, the methyl ester of p-bromo-benzoic acid separates as a white, usually friable powder. It is filtered off, washed with cold water until free from alkali, and dried upon a porous plate or in a vacuum-desiccator for the purpose of finally determining its melting-point (78–79°). After the powder has been washed, crystallize it from alcohol, methyl alcohol or acetone. A solution saturated at 15–20° is prepared, filtered, mixed with an equal volume of water, rapidly stirred and set in ice. From alcohol the methyl ester of p-bromo-benzoic acid is obtained in the form of shining leaflets, from methyl alcohol in fine needles. From hot benzene solution the ester crystallizes upon addition of petroleum ether in shining leaflets. Cold alcohol dissolves it to the extent of 2.3 per cent., but it is more easily soluble in acetone.

Methyl alcohol in aqueous solution can be determined practically quantitatively by this reaction. By the introduction of the bromo-benzoyl radical into the molecule of methyl alcohol, a compound of low molecular weght (CH_4O = 32) is converted into one of high molecular weight ($C_6H_4Br.COOCH_3$ = 215). The theoretical yield of ester from 1 gram of methyl alcohol is 6.75 grams. Therefore the delicacy of the test is very great. Even quantities of only 0.05–0.1 gram of methyl alcohol in dilutions of 1 : 200 to 1 : 400 still give weighable amounts of the methyl ester of p-bromo-benzoic acid. By employing the following

[1] W. Autenrieth: Detection of Methyl Alcohol as the Methyl Ester of p-Bromo-Benzoic Acid. Arch. d. Pharm. 258 (1920), 1.

procedure, 55–60 per cent. of the theoretical quantity of ester may be obtained. Add to the given aqueous solution in a glass-stoppered flask 10–15 cc. of sodium hydroxide solution, heat in a water-bath until the contents of the flask are at 40–45°, then add 2–3 grams of ground p-bromo-benzoylchloride, and shake vigorously until cold. The reaction of the mixture should remain alkaline. Otherwise add 2–3 cc. more of sodium hydroxide solution, shake again and test with litmus paper. Then transfer the mixture, rinsing the flask with 10–15 cc. of ether, to a rather small separatory funnel without loss. After shaking with the ether, withdraw this solvent into a dry flask, taking the greatest care not to get in even a drop of the aqueous solution. Extract with a fresh portion of 10 cc. of ether and also transfer this ether extract without loss to the flask. Now add to the ether solution 2–3 pieces of anhydrous calcium chloride, allow to stand for some time, pour through a dry filter, rinsing out the flask with a little ether, collect the filtrate in a small, dry, weighed flask, evaporate the ether solution in a vacuum desiccator (Fig. 13), and dry to constant weight. In most cases the methyl ester of p-bromo-benzoic acid will be obtained in well crystallized form and have the correct melting-point. For the purpose of recrystallizing the weighed ester, dissolve it in the calculated quantity of cold alcohol, keeping in mind that it is soluble to the extent of 2.3 per cent. Then add an equal volume of water and allow the mixture to stand in ice. Usually the pure ester crystallizes at once and its melting-point may be redetermined.

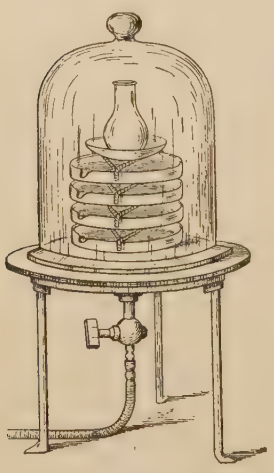


FIG. 13.—Vacuum bell-desiccator.

Example.—The materials taken were: 0.1 gram of methyl alcohol; 20 cc. of water; 15 cc. of sodium hydroxide solution; and 2.5 grams of p-bromo-benzoyl-chloride. A weight of 0.35 gram of ester was obtained equivalent to 53 per cent. of the theory in a methyl alcohol concentration of 1:350.

Detection of Methyl Alcohol in Parts of Cadavers and Urine with p-Bromo-benzoylchloride

Since ammonia and ammonium salts as well as volatile phenols, such as phenol and p-cresol, are always formed in the putrefaction of parts of cadavers and also give crystalline compounds with p-bromo-benzoylchloride in presence of sodium hydroxide solution, that is, p-bromo-benzamide, $C_6H_4Br.CO.NH_2$, and the phenyl ester of p-bromo-benzoic acid, $C_6H_4Br.COOC_6H_5$, as well as the cresyl ester, parts of cadavers and urine should first be strongly acidified and then subjected to distillation to prevent ammonia from passing over. If a portion of the distillate gives a red color when warmed with Millon's reagent, phenols are present. To hold back the latter, render the rest of the distillate strongly alkaline with sodium hydroxide solution and distil once more. The distillate now obtained may be used for the detection of methyl alcohol with p-bromo-benzoylchloride and sodium hydroxide solution.

Identification of Methyl Ester of p-Bromo-benzoic Acid.—1. By its anise-like odor; 2. By its sharp melting-point (78–79°); 3. By the determination of bromine according to the Carius or the fusion method (37.21 per cent.); 4. By converting it by means of strong aqueous or alcoholic ammonia into p-bromo-benzamide, crystallizing in leaflets and having the melting-point 188°.

Notes.—The bell-desiccator[1] for vacuum-drying shown above (Fig. 13) has rendered excellent service in evaporating ether and chloroform solutions, as well as in drying the methyl ester of p-bromo-benzoic acid and many other substances *in vacuo* to constant weight. In using concentrated sulphuric acid as a drying-agent only enough acid to cover the bottom of the flat dishes should be taken.

Formic Acid

Formic acid, H.COOH, hardly ever comes up as a poison. If this acid in concentrated form gets upon the skin it may produce blisters and even cause necrosis. Injected under the skin, even small doses of formic acid give rise to pain, hyperemia and inflammation (R. Kobert). Formic acid possesses special toxicological interest for the reason that it occurs in the urine in larger quantity during methyl alcohol poisoning. According to v. Jaksch and v. Rokitansky, every normal human urine contains small quantities of volatile fatty acids, that is principally acetic acid together with a little formic acid and probably also butyric acid. Salkowski[2] found an increased amount of formic acid in leukemia. According to Autenrieth,[3] formic acid may be regarded as a normal constituent of human urine, for he detected at least traces of this acid in all urines examined. The fluctuation in the percentage of formic acid in the urine of the same individual during a period of 4 months was not very great. Upon the average a person eliminates in the urine 0.251 gram of formic acid in the course of 24 hours. After a dose of methyl alcohol the formic acid-content of the urine shows a considerable increase. A man, who had taken 80 grams of pure methyl alcohol highly diluted during a period of 8 days, eliminated in this time 5.3 grams more formic acid than he did normally. The formic acid-content of the urine is also strongly increased after taking a salt of formic acid. After a dose of 20 grams of sodium formate, distributed over 2 days, the formic acid output was increased about 2.4 grams, that is, 18 per cent. of the formic acid taken escaped oxidation in the body of the individual (W. Autenrieth). In toxicologico-chemical investigations the mere qualitative detection of formic acid in urine is not sufficient basis for a final decision in a case of suspected methyl alcohol poisoning. A quantitative determination of formic acid in the urine of the person poisoned is absolutely essential before an opinion not open to question can be rendered.

[1] These vacuum desiccators can be procured from the firm of Dr. Bender and Hobein in Munich, Germany.

[2] E. Salkowski: Formation of Volatile Fatty Acids in Ammoniacal Fermentation of Urine. Zeitschr. f. physiol. Chem. 13 (1889), 264.

[3] W. Autenrieth: Formic Acid Content of Urine Normally and after Administration of Different Substances. Münch. med. Wochenschr. 1919, No. 31, page 862.

Quantitative Estimation of Formic Acid

Formic acid is slightly volatile with steam and actually passes over with more difficulty than does acetic acid. Ordinarily it is determined quantitatively by the method of Scala-Lieben.[1] This is based upon the reduction of mercuric chloride by formic acid to mercurous chloride which is finally weighed:

$$2HgCl_2 + H.COOH = Hg_2Cl_2 + CO_2 + 2HCl.$$

This method requires rather long warming with a considerable excess of mercuric chloride. Since various aldehydes volatile with steam, such as formaldehyde, acetaldehyde and furfural, in addition to formic acid will also cause reduction of mercuric chloride with heat, separation of formic acid from aldehydes present becomes necessary. The best procedure for this purpose is to add calcium carbonate to the distillate and evaporate.

Procedure.—Add 30 cc. of 25 per cent. phosphoric acid and a few pieces of pumice-stone to 300 cc. of the given urine in a 750 cc. distilling flask. Use a still-head and conduct distillation at a very vigorous boil. Many urines froth badly when they begin to boil and for this reason only a small flame should be used during the first 10 minutes of distillation. This difficulty, however, is soon over and then the liquid boils quietly. Collect the distillate in a graduate. When about 300 cc., but no more, have passed over and about 30 cc. remain in the flask, stop distillation and add 30 cc. of water to the flask. Again distil off the same volume and repeat until the distillate fails to react acid with litmus paper. This point is usually reached when 1500–2000 cc. of distillate have passed over. Stir an excess of calcium carbonate with water and add to the distillate. Bring at once to a boil, then evaporate in a flat dish upon the water-bath to 20–30 cc. and filter. Rinse dish and filter several times with a little hot water. Transfer the entire filtrate to an Erlenmeyer flask and add 50–60 cc. of cold saturated mercuric chloride solution. Shake frequently and heat for 5–6 hours in the water-bath under a reflux. Then add according to the amount of precipitate 2–3 cc. of 20 per cent. hydrochloric acid, shake and warm 10 minutes longer in the water-bath. Collect mercurous chloride upon a filter dried at 100–110°, or in a Gooch crucible, wash with water until free from chlorine, then with alcohol followed by ether, dry for 1 hour at 100–110° and weigh. To calculate the quantity of formic acid, multiply the weight of mercurous chloride precipitate by 0.0977.

Fincke[2] adds 3–5 grams of crystallized sodium acetate to the neutral or faintly acid solution of formic acid, the volume of which in the case of rather small quantities should be 50–100 cc. and for larger quantities, over 50 milligrams of formic acid, 100–300 cc., and a quantity of mercuric chloride at least 15 times that of the formic acid. The entire mixture is put into an Erlenmeyer flask, provided with a rubber stopper and a glass tube 30–40 cm. long for a reflux, and

[1] A. Scala: Quantitative Estimation of Formic Acid in Presence of Acetic Acid and Butyric Acid. Gazz. chim. Italian. 20 (1860), 393; A. Lieben: Estimation of Formic Acid. Monatsh. f. Chem. 14 (1893), 747.

[2] H. Fincke: Contribution to the Estimation of Formic Acid in Foods. Zeitschr. f. Nahrungs- u. Genussm. 22 (1911), 88.

heated for 2 hours in an actively boiling water-bath. Mercurous chloride is filtered hot and otherwise treated as described above.

Notes.—Use of a caustic alkaline solution instead of calcium carbonate for the neutralization of formic acid is not permissible, because aldehydes are held back in the form of aldehyde resins and formaldehyde present may possibly be decomposed to methyl alcohol and formic acid.

Estimation of Formic Acid in Presence of Salicylic Acid

If the solution of a salicylate is heated with mercuric chloride solution in presence of sodium acetate, as in Fincke's method of estimating formic acid, turbidity appears and gradual separation of a compound of mercury and salicylic acid takes place. This separation may be prevented by addition of sodium chloride, so that formic acid can be estimated by means of mercuric chloride in presence of salicylic acid. Of course too large quantities of sodium chloride affect the accuracy of the estimation of formic acid.

BITTER ALMOND WATER AND OIL OF BITTER ALMONDS

Bitter almond water (Aqua Amygdalae Amarae of the Pharmacopoeia) contains hydrocyanic acid. Only a small portion of this acid, however, is free so that it can be precipitated by silver nitrate solution. The greater part is combined as the cyanohydrin of benzaldehyde which does not react with silver nitrate. But potassium hydroxide solution will decompose this compound:

$$C_6H_5.CH(OH)CN + KOH = KCN + H_2O + C_6H_5.CHO.$$

Pure benzaldehyde, also called hydrocyanic acid-free oil of bitter almonds, is not poisonous. It is oxidized to benzoic acid in the body and eliminated in the urine, partly as that acid and partly as hippuric acid, after conjugation with glycocoll (amino-acetic acid):

$$(\alpha) \qquad C_6H_5.CHO + O = C_6H_5.COOH$$

$$(\beta) \quad \underset{\text{Benzoic acid}}{C_6H_5.COOH} + \underset{\substack{\text{Glycocoll furnished by the organism}}}{H_2N.CH_2.COOH} = \underset{\text{Hippuric acid}}{C_6H_5.CO.NH.CH_2.COOH}.$$

Ordinary commercial oil of bitter almonds contains hydrocyanic acid and is poisonous in proportion to the quantity of this acid present. To test for hydrocyanic acid, shake a few cc. of oil of bitter almonds with 10 cc. of potassium hydroxide solution, separate the latter from the oil, and make the Prussian blue test.

When oil of bitter almonds is mixed with other material, distil with steam from a solution acidified with tartaric, or dilute sulphuric acid, and test the first part of the distillate for hydrocyanic acid. If benzaldehyde is present, the distillate at the same time will be milky and have the characteristic odor of that compound. Distil until the drops of water are perfectly clear. Benzaldehyde may be detected with certainty, and at the same time distinguished from the highly poisonous nitro-benzene which has a somewhat similar odor, by adding a few drops of potassium hydroxide solution to the milky distillate, to combine with any hydrocyanic acid, and extracting with ether. The ether upon evaporation

will deposit benzadehyde as oil-drops which can be positively identified by conversion into benzoic acid. Heat the oil-drops for a few minutes in a small flask provided with a reflux with about 10 cc. of potassium dichromate solution and a little dilute sulphuric acid. Cool, extract with ether, and evaporate the ether solution in a glass dish. When the material contains benzaldehyde, this residue will consist of benzoic acid. This substance may be further identified by its melting-point (120–121°), its property of subliming, and the test with ferric chloride solution.[1]

To test for nitrobenzene in a distillate that has an odor like that of bitter almonds, shake with tin and hydrochloric acid (see page 70) and then test for aniline.

With regard to the "*Estimation of minute quantities of hydrocyanic acid colorimetrically*" and the "*Examination of bitter almond water*," see Chapter V, pages 540 and 543 of this book.

CARBON DISULPHIDE

Carbon disulphide, CS_2, is a colorless liquid having a characteristic odor and a high index of refraction. As the following table shows, there is some difference of opinion with regard to the solubility of carbon disulphide in water:

Temperature	1000 cc. of water dissolve	
13–14°	2.03	parts (Page)
15–16°	1.81	parts (Chancel; Parmentier)
15–16°	2.0–3.0	parts (Ckindi)
15–16	3.5–4.52	parts (Péligot)

Carbon disulphide is miscible in all proportions with absolute alcohol, ether, ethereal and fatty oils.

Toxic Action.—Carbon disulphide administered internally has a very poisonous action upon the blood, causing especially decomposition of red blood-corpuscles. Even inhalation of carbon disulphide vapor frequently occasions severe poisoning. Carbon disulphide was formerly considered a typical producer of methaemoglobin but recent investigations have not confirmed this opinion. It has a very injurious action upon the red blood-corpuscles, causing haemolysis. R. Kobert (Intoxikationen) states that its power of dissolving lipoids is responsible for its injurious action upon the blood and the central nervous system. Harmsen[2] has recently come to practically the same conclusion. He considers carbon

[1] Dissolve the residue in a small quantity of water and neutralize benzoic acid by heating the solution to boiling with excess of calcium carbonate. Filter and add a few drops of ferric chloride solution. If benzoic acid is present, a flesh-colored precipitate of basic ferric benzoate will appear. Tr.

[2] E. Harmsen: Carbon Disulphide Poisoning in Factories. Vierteljahrsschr. f. gerichtl. Med. usw. 30 (1905), 422.

disulphide a powerful blood-poison, because it decreases the number of red blood-corpuscles and the quantity of haemoglobin and brings about a leucocytosis.[1]

Detection of Carbon Disulphide

Carbon disulphide distils very slowly with steam. Consequently the second or third fraction of the distillate should be used in testing for this substance. If 40 cc. are distilled from 100 cc. of water, containing 2 drops of carbon disulphide, the following 10 cc. will give a distinct test. If the quantity of carbon disulphide is small, it will remain in solution. Such a solution does not have a strong odor. It is not impossible that the aqueous solution of carbon disulphide contains dithio-carbonic acid:

$$CS_2 + H_2O = CS(SH)(OH).$$

Carbon disulphide may be recognized in the distillate by the following tests:

1. Lead Acetate Test.—Add a few drops of lead acetate solution to the liquid containing carbon disulphide. It will cause neither a precipitate (distinction between CS_2 and H_2S) nor a color. Add excess of potassium hydroxide solution and boil. A black precipitate (PbS) will appear. This is a very delicate test.

2. Sulphocyanate Test.—Heat an aqueous solution of carbon disulphide for a few minutes with concentrated ammonium hydroxide and alcohol. Ammonium sulphocyanate (H_4NSCN) is formed together with ammonium sulphide. Concentrate this solution upon the water-bath to about 1 cc. and acidify with dilute hydrochloric acid. Add a drop of ferric chloride solution and a deep red color will appear. This test will show even traces of carbon disulphide, for example, 0.05 gram in 1 cc. of water.

$$(\alpha) \qquad 4NH_3 + CS_2 = (H_4N)SCN + (H_4N)_2S,$$
$$(\beta) \quad FeCl_3 + 3(H_4N)SCN = Fe(SCN)_3 + 3(H_4N)Cl.$$

3. Xanthogenate Test.—Shake a few cc. of a distillate containing carbon disulphide for several minutes with about 3 times its volume of saturated solution of potassium hydroxide in absolute alcohol. Faintly acidify the solution with acetic acid and add 1–2 drops of copper sulphate solution. If carbon disulphide is present, a brownish black precipitate of cupric xanthogenate, will appear. This will

[1] Leucocytosis is a temporary increase in the number of white blood-corpuscles (leucocytes) as compared with the number of red blood-corpuscles. Normally there are about 350 red to 1 white blood-corpuscle, whereas in leucocytosis the proportion is 20:1.

soon change to a yellow, flocculent precipitate of cuprous xanthogenate, $CS(SCu)(OC_2H_5)$.

Quantitative Estimation of Carbon Disulphide in Air

Inhalation of air containing carbon disulphide has frequently given rise to chronic poisoning. Individuals thus affected have usually been workmen in rubber factories. Consequently experiments have been made to determine the maximum quantity of carbon disulphide air may contain without injury to health. The results of these investigations may be summarized as follows:

No.	CS_2 in mgrs. per liter of air	Result
1.	0.5–0.8	No injurious effect.
2.	1.3	Slight uneasiness after several hours.
3.	3.4	Uneasiness in 30 minutes.
4.	6.0	Uneasiness in 20 minutes.
5.	10.0	Paralysis attended by after-effects lasting several days.

The exact danger limit for persons obliged to live for weeks at a time in an atmosphere containing carbon disulphide should be placed below 3 milligrams in a liter of air. Air in factories, where operatives work in presence of carbon disulphide vapor, should never exceed this limit. Since experiments have shown that 93–96 per cent. of the carbon disulphide breathed was exhaled unchanged, an exceedingly small quantity is capable of producing toxic symptoms.

Procedure.—To determine quantitatively the amount of carbon disulphide in the air, place a saturated alcoholic solution of potassium hydroxide in a Péligot absorption-tube and draw through this solution 10–20 liters of air containing carbon disulphide vapor. A quantitative formation of potassium xanthogenate, $CS(SK)(OC_2H_5)$, will take place. At the end of the experiment dilute the contents of the receiver with 96 per cent. alcohol and bring the volume to 50 cc. Measure an aliquot portion of this solution and dilute with water. Faintly acidify the solution with acetic acid and remove excess of acid with acid sodium carbonate. Add freshly prepared starch solution and 0.1 n-iodine solution until there is a permanent blue color.

Explanation.—Iodine converts potassium xanthogenate according to equation (I) into ethylxanthogen disulphide:

$$\text{I. } I_2 + 2KS.CS.OC_2H_5 = 2KI + \begin{vmatrix} S.CS.OC_2H_5. \\ S.CS.OC_2H_5 \end{vmatrix}$$

Rupp and Krauss[1] think the action of iodine upon potassium xanthogenate is expressed by equation (II):

$$\text{II. } 2KS.CS.OC_2H_5 + I_2 + H_2O = KS.CS.SK + 2C_2H_5.OH + 2HI + S.$$

Both equations require the same quantity of iodine, that is, 2 atoms for 2 molecules of xanthogenate. A difference therefore in the mechanism of the reaction has no influence upon the combining relations of the iodine, and the method is applicable to the quantitative determination of xanthogenate.

1000 cc. of 0.1 n-iodine solution, containing 0.1 gram-atom of iodine, correspond to 0.1 gram-molecule of CS_2 = 7.6 grams.

Preservation of Cadaveric Material for Toxicological Examination

Various liquids used to preserve cadaveric material for later toxicologica examination, such as sulphur dioxide, chlorine water, calcium chloride, other metallic salts, and alcohol itself, possess certain disadvantages and often fail to meet the requirements. A satisfactory preserving solution should make it impossible for histological or chemical changes to take place in tissues. It should not introduce any foreign substance into the material to be preserved either itself or impurities that it may contain. According to Mameli,[2] liquid air fulfils these requirements. Every putrefactive process, as well as every chemical action, is checked by its low temperature (−190°). It prevents loss of poisons that are easily volatile or alterable, such as carbon monoxide and hydrocyanic acid. Yellow phosphorus is not oxidized when kept for some time in liquid air. For this reason it was found possible to get a positive Mitscherlich test in the case of a rabbit to which phosphorus had been given. The stomach and contents were preserved for 40 hours in liquid air. An additional advantage of this method is that cadaveric material preserved in liquid air becomes so brittle that it can be ground in a mortar.

Grigorjew[3] has recommended formalin as a preserving solution for cadaveric material and Barth[4] has carried on extensive experiments upon its use for this purpose. The material used for examination was fresh beef liver finely chopped. It was covered with the preserving fluid and allowed to stand at room temperature for varying lengths of time in glass jars covered with parchment paper and kept where it was light. The poisons selected were the alkaloids, morphine, strychnine, atropine and veratrine; and arsenic, phosphorus, potassium cyanide

[1] E. Rupp and L. Krauss: Iodimetric Estimation of Copper as Cuprous Xanthogenate. Ber. d. Deutsch. chem. Ges. 35 (1902), 4157.

[2] E. Mameli: Use of Liquid Air in Toxicology. Boll. della Società Medico-chirur. di Pavia 1910.

[3] A. Grigorjew: Preservation of Parts of Cadavers. Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw. 29, 79.

[4] Barth: Pharm. Journ. 44 (1905), 51.

and phenol. At the same time parallel experiments were made with distilled water, alcohol, and glycerol. In detecting alkaloids, the Stas-Otto process was used. From the results obtained in these experiments, Barth drew the following conclusions. Formalin is much to be preferred to alcohol as a preservative because material kept in it gives purer extracts. The free alkaloids pass in purer condition into the ether and chloroform extracts and do not have to be converted into salts for purposes of purification. When formalin was used as a preservative, 0.02 gram of alkaloid could be detected even after 12 months, whereas when alcohol was used no test for 0.02 gram of atropine was given after the same period of time. Phosphorus is very well preserved by formalin. Potassium cyanide and carbolic acid, however, did not give as favorable results. No test for hydrocyanic acid was given in the case of 0.02 gram of potassium cyanide in 5 and 10 per cent. formalin solution, although the material was well-preserved.[1] But a test for hydrocyanic acid was obtained where liver, containing 0.2 gram of potassium cyanide, was preserved in 1 per cent. formalin solution, because the quantity of formalin was not sufficient to combine chemically with the entire quantity of potassium cyanide present. The outcome of the experiments with phenol was the same. Phenol could not be detected with ferric chloride, when less than 0.5 per cent. was in 10 per cent. formalin solution, not even immediately after mixing the two solutions. These two substances must react as shown in the following equation:

$$CH_2O + 2C_6H_5.OH = H_2O + CH_2(C_6H_4.OH)_2.$$

On the other hand, ferric chloride gave a test when larger quantities of phenol were used.

Although formalin possesses many advantages over alcohol, it is not always to be recommended as a preservative of cadaveric material that is to be examined for poisons. Under no circumstances should formalin be used, when it is a matter of using the material for the detection of hydrocyanic acid, carbolic acid or other volatile phenols.

[1] The addition-product, $CH_2(OK)(CN)$, may be formed by the interaction of formaldehyde and potassium cyanide.

CHAPTER II

NON-VOLATILE POISONS

Organic Substances Non-volatile with Steam from Acid Solution but Extracted by Acidulated Alcohol[1]

Put a portion of finely chopped material into a large flask and thoroughly mix with 2–3 times as much absolute alcohol.[2] Add enough tartaric acid solution to give the mixture an acid reaction after shaking. Laboratory experiments usually require 20–30 drops of 10 per cent. tartaric acid solution. Avoid a large excess of tartaric acid, since it may act as an objectionable impurity in the ether extract, owing to its solubility in that solvent. Connect the flask with a glass tube (80–100 cm. long) serving as a reflux. Frequently shake and heat 10–15 minutes upon the water-bath. In the extraction of a large quantity of material from a cadaver, connect the flask with an upright Liebig condenser used as a reflux (Fig. 14). Cool the flask contents and filter to remove fat as completely as possible. Wash the residue with alcohol. Evaporate the filtrate, which should have an acid reaction,[3] to a thin syrup in a glass dish upon the water-bath. Thoroughly mix this residue with 100 cc. of cold water. Usually this causes an abundant separation of fat and resinous matter, especially when parts of a cadaver are examined. Filter through a moistened creased paper and evaporate the filtrate to dryness, or to a syrup, upon the water-bath. Thoroughly mix this residue with absolute alcohol. As a result of this treatment, a

[1] Artificial mixtures for laboratory practice should be examined according to the method outlined above. They may consist of dog-biscuit, meat, comminuted organs (liver, kidneys, spleen, etc.), sausage meat, or other materials mixed with any of the poisons of this group. The same systemetic procedure should be followed in examining soup, milk, vegetables, and potatoes for these poisons.

[2] Commercial alcohol usually contains basic compounds, the presence of which is objectionable. They should be removed by adding tartaric acid to the alcohol and distilling. Alcohol should not be used in toxicological analysis, unless an actual test has shown it to be free from such impurities. Tr.

[3] If this filtrate is not acid (litmus paper test), it should be acidified with a few drops of tartaric acid solution.

whitish substance, more or less viscous or slimy, usually remains undissolved. This residue, consisting chiefly of protein substances (albumin, albumoses and peptones), dextrin-like compounds, and in part also of inorganic salts, frequently becomes granular upon standing. Tartrates of the alkaloids and other organic poisons are dissolved. The larger the quantity of absolute alcohol used, the more complete is the precipitation of those substances that interfere more or less with the detection of organic poisons. Again evaporate the filtered alcoholic solution upon the water-bath and dissolve the residue in about 50 cc. of water. If the solution is not perfectly clear, filter through a moistened paper.

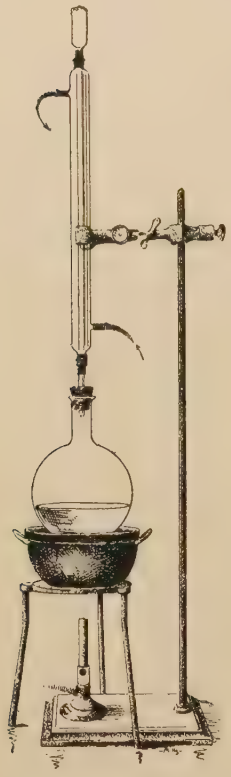


Fig. 14.—Liebig condenser as reflux.

The result of this procedure is a solution containing alkaloidal tartrates and other organic substances belonging to this group. The solution should have an acid reaction and be practically free from protein substances, fat, resinous bodies, and coloring-matter. If the solution fulfils these requirements, it is ready to be examined for organic poisons according to the "Stas-Otto" method. The utmost care should be taken in preparing this solution, for definite conclusions cannot be drawn from the uncertain tests given by impure material.

This aqueous acid solution should be examined according to the Stas-Otto process in the following manner:

A. Thoroughly extract the acid solution several times with ether. This solvent will dissolve: free acids and acid compounds, such as picric acid, salicylic acid, veronal; neutral substances, such as acetanilide and phenacetine; and very weak bases, such as antipyrine, caffeine, colchicin and narcotine. But only traces of the last-mentioned substances pass from the tartaric acid solution into the ether.

B. The acid aqueous solution remaining after extraction with ether should be made strongly alkaline with sodium hydroxide solution and again extracted with ether. Sodium hydroxide sets

free basic substances, that is, most of the alkaloids with the exception of phenolic bases, such as morphine and apomorphine which remain in the aqueous solution as sodium compounds. Ether dissolves these liberated bases.

C. The aqueous solution alkaline with sodium hydroxide must be changed to one alkaline with ammonia. This can be brought about by adding ammonium chloride. Excess of sodium hydroxide is combined and any sodium compounds of phenolic bases that may be present are at the same time decomposed.[1] Some analysts, however, recommend neutralizing the sodium hydroxide solution with dilute hydrochloric acid and then making alkaline with ammonia. This aqueous ammoniacal solution should then be extracted as follows:

(α) Ether[2] will extract apomorphine and traces of morphine.

(β) Hot chloroform, or according to the Stas-Otto process pure amyl alcohol, will extract morphine and narceine from the aqueous solution. This chloroform extract may also contain colchicin, caffeine and antipyrine, as well as those substances of *A* and *B* that are only slightly soluble in ether. Kippenberger acidifies the alkaline aqueous solution, after extraction with ether, with dilute hydrochloric acid. He then renders this solution alkaline with sodium bicarbonate instead of with ammonia and extracts morphine, narceine, and the other substances that belong here, with a hot mixture consisting of 90 volumes of chloroform and 10 volumes of absolute alcohol. The author highly recommends this hot alcohol-chloroform mixture for the extraction of morphine.

Examination of a Powdered Mixture Containing Cane-Sugar or Milk-Sugar

When the material is a powder mixed with cane-sugar or milk-sugar, it is usually possible, after the aqueous solution has been acidified with tartaric acid, to extract direct. The acid solution is

[1] Sodium morphinate, for example, is decomposed in the following manner:

$$C_{17}H_{18}O_2N(ONa) + (H_4N)Cl = NaCl + NH_3 + C_{17}H_{19}O_3N.$$

[2] This ether extraction (α) of the ammoniacal solution is not necessary, unless the ether extracts of the acid aqueous solution, or of this solution after it has been made alkaline with sodium hydroxide, have a red or violet color due to colored decomposition products of apomorphine, that is, when these colors point to the presence of apomorphine.

filtered hot and examined for poisons by the Stas-Otto method according to the procedure outlined above under *A*, *B* and *C*.

Examination of Beer, Wine and Infusions of Cocoa, Coffee and Tea

In the examination of such material, which is frequently brought to the forensic chemist to be tested for poison, the process outlined above may be considerably abridged. Acidify the material with aqueous tartaric acid solution, if necessary, and evaporate in a glass dish upon the water-bath. Treat the residue thoroughly with absolute alcohol and filter. Evaporate the filtrate upon the water-bath and dissolve the residue in tepid water. Filter this solution, if necessary, and then examine according to the Stas-Otto process.

A. Preparation and Examination of Ether Extract of Aqueous Tartaric Acid Solution

Thoroughly extract the acid aqueous solution (see process of preparation described above) 2–3 times with ether, using each time about the same quantity of solvent. Employ a separating funnel for this purpose (Fig. 15). Pour the combined ether extracts into a dry flask loosely stoppered. If the solution stands for 1–2 hours at rest, a few drops of water usually settle out. Decant the ether solution and pour through a dry filter. Slowly evaporate this solution in a small glass dish upon a water-bath previously heated slightly above 35°. **Do not have gas burning during this operation!** Examine the residue as described below. An excellent method of evaporating ether consists in setting a small glass dish (8–10 cm. in diameter) upon a hot water-bath and dropping the filtered ether extract into it as fast as the solvent evaporates. Thus a large quantity of extract may be evaporated in a small dish. The advantage of this method is the ease with which the residue can be removed for the various tests. The residue is usually quite small and it is not advisable to have it distributed over too large a surface. Larger quantities of ether solution are distilled in a small dry flask upon the water-bath until about 10 cc. remain. The latter may then be evaporated upon a small watch-glass.

Examine the residue from the ether extract for the following substances:

Picrotoxin Picric Acid Acetanilide Veronal Caffeine
Colchicin Salicylic Acid Phenacetine Antipyrine Cantharidin

The feebly basic substances, antipyrine and caffeine, appear only in small quantity in the ether extract of the tartaric acid solution. By far the greater part of these two substances is extracted by ether or chloroform from the aqueous solution after it has been rendered alkaline with sodium hydroxide or ammonia. The very feebly basic colchicin, which is only slightly soluble in ether, also appears

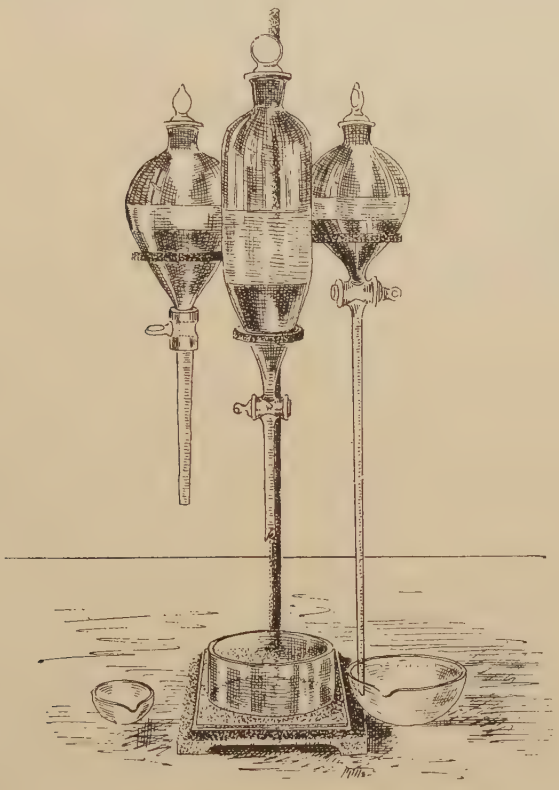


FIG. 15.—Separating funnels and glass crystallizing dishes.

mainly in the chloroform extract of the aqueous solution after it has been rendered alkaline with ammonia.

Evaporation of the ether extract, even in the absence of members of the group, usually leaves a more or less viscous residue, containing tartaric and lactic acid as well as fatty, resinous and colored substances. This is particularly the case in analyses of cadaveric

material. Moreover, ether extracts certain metallic salts from aqueous solutions, for example, mercuric cyanide[1] and chloride.

First, note the general appearance and taste of the residue. Then examine it with a microscope. Very definite conclusions as to the presence or absence of certain substances can frequently be drawn. A very bitter residue should be examined especially for picrotoxin and colchicin. If there is a pronounced yellow color, the examination should include picric acid also. Veronal is colorless and has a pleasantly bitter taste. The same is true of caffeine. A tasteless or only faintly bitter residue probably does not contain these substances and should be examined for acetanilide, antipyrine, phenacetine and salicylic acid.

The residue from evaporation of the ether extract may contain the following substances:

Picrotoxin.—Usually a thick syrup that gradually solidifies and becomes crystalline. It has an intensely bitter taste.

Colchicin.—Yellowish, amorphous residue that does not become crystalline. Tastes intensely bitter. Dissolves in water with a yellow color.

Picric Acid.—Usually appears as a syrup that gradually solidifies and becomes crystalline. Tastes very bitter. Residue intensely yellow, giving yellow aqueous solutions.

Acetanilide.—Leaflets or flat needles. Has a faint burning taste but is not bitter.

Phenacetine.—Inodorous and tasteless leaflets and small needles.

Antipyrine.—Residue a syrup that rarely crystallizes. Tastes mildly bitter. Very easily soluble in water.

Salicylic Acid.—Crystallizes in small needles. Has a harsh and at the same time sweet, acidulous taste.

Veronal.—Leaflets and needles having a bitter taste.

Caffeine.—Residue composed of small needles frequently in radiating clusters. Tastes mildly bitter.

[1] To some extent ether will extract mercuric cyanide from a tartaric acid solution that is not too dilute. For example, it will remove appreciable quantities from 100 cc. of 0.1 per cent. mercuric cyanide solution but the extraction will not be complete. The solution after five extractions will still give a distinct test for mercury. Ether will not remove even a trace of mercuric cyanide from 0.01 per cent. solution. To test for cyanide, add ammonium sulphide solution to the ether residue. This will precipitate mercuric sulphide and the filtrate will contain ammonium sulphocyanate (see hydrocyanic acid, page 30).

The residue from the ether extract evaporated upon a watch-glass should not be heated upon the water-bath longer than necessary, for various substances becomes viscous and salicylic acid, if present, may volatilize. Remove the watch-glass as soon as the odor of ether is gone. Scrape together the cold residue by means of a platinum or nickel spatula, or clean knife-blade, dividing it into several portions and testing for the poisons that may appear.

The author has frequently found the apparatus shown in Fig. 16 useful in distilling or evaporating larger quantities of liquid *in vacuo*. The vacuum apparatus consists of a porcelain basin, suspended in a water-bath and tightly connected by a broad, well-ground edge with a vacuum-bell. A tight connection

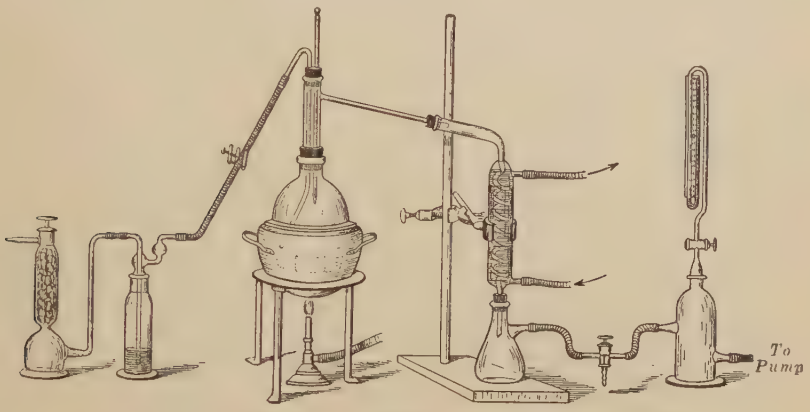


FIG. 16.—Apparatus for distillation or evaporation in vacuo.

can be secured by using a rubber ring. But the latter is unnecessary if the porcelain basin and vacuum-bell fit well. The liquid to be distilled or evaporated may be placed in the porcelain basin itself, or in any containing-vessel, such as a porcelain dish, watch-glass, beaker, etc., set in the basin.

PICROTOXIN

Picrotoxin, $C_{30}H_{34}O_{13}$, the poisonous principle of Cocculus indicus, the fruit of Menispermum Cocculus, is present to the extent of 5 per cent. Owing to its stupefying action upon fish, it is employed in catching them, and because of its very bitter taste is said to have found use as a substitute for lupulin.

Properties.—Picrotoxin crystallizes from hot water in long, colorless needles melting at 199–200°. It dissolves with difficulty in cold water but considerably more easily in hot water or alcohol. It is only slightly soluble in ether but freely soluble in chloroform, amyl alcohol and glacial acetic acid. Its alcoholic solu-

tion is neutral and laevo-rotatory. Picrotoxin has a very bitter taste. It is soluble in caustic alkalies and aqueous ammonia, forming unstable, salt-like compounds that do not crystallize. Picrotoxin has therefore the character of a weak acid. After prolonged boiling with considerable benzene, it is decomposed into picrotoxinin and picrotin. The former passes into solution but picrotin is almost completely insoluble:

$$\underset{\text{Picrotoxin}}{C_{30}H_{34}O_{13}} = \underset{\text{Picrotoxinin}}{C_{15}H_{16}O_6} + \underset{\text{Picrotin}}{C_{15}H_{18}O_7}.$$

Chloroform brings about this cleavage even more easily.

On the other hand, if picrotoxinin and picrotin in molecular proportions are dissolved in hot water, picrotoxin crystallizes out as the solution cools. Treated with bromine direct or dissolved in water or ether, picrotoxin is first split into picrotoxinin and picrotin. The former is immediately converted into monobromo-picrotoxinin, $C_{15}H_{15}BrO_6$, but picrotin remains almost unchanged. Monobromo-picrotoxinin is soluble with difficulty in water but is reduced by zinc dust and acetic acid to picrotoxinin. Picrotoxinin has a very poisonous action, whereas picrotin is almost non-toxic.

Meyer and Bruger[1] regard picrotoxin as a complex of the two compounds, picrotin and picrotoxinin, crystallizing together in definite but not molecular proportion, and not as a molecularly constituted chemical compound.

Toxic Action.—Picrotoxin is a powerful convulsive poison, standing in its action between cicutoxin and strychnine. Its absorption takes place easily. A few minutes after the poison has been taken, the first symptoms of intoxication appear (emesis, diarrhoea, dizziness, enlargement of the pupils, epileptic or tetanic convulsions, accompanied by peculiar cries, flow of saliva, sweating and delirium). The poison in part is eliminated unchanged in the urine which should first be tested in case of poisoning by picrotoxin. Kobert has described a case of poisoning, the course of which was extremely violent. A man, who had taken tincture of Cocculus indicus in small doses for a week and then a larger dose at one time, was taken with dizziness, nausea and very soon with a convulsion that threw him to the floor. Tonic and clonic convulsions followed with loss of consciousness and 30 minutes after the dose was taken death ensued. The lethal dose is small. In one case death resulted from 2.4 grams of the seed.

Detection of Picrotoxin

Picrotoxin, picrotoxinin and picrotin act as reducing agents.

1. Fehling's Test.—Dissolve picrotoxin in a small test-tube, using 10–20 drops of very dilute sodium hydroxide solution. Add a few drops of Fehling's solution[2] and warm but do not shake. A red or yellowish red precipitate gradually forms and settles to the bottom. If the ether residue, not too little of which should be taken, fails to

[1] R. J. Meyer and P. Bruger: Contribution to the Knowledge of Picrotoxin. Ber. d. Deutsch. chem Ges. 30 (1898), 2958.

[2] Fehling's solution heated by itself should not give a precipitate of cuprous oxide.

give a clear solution in very dilute sodium hydroxide solution, filter through moistened paper and examine the filtrate with Fehling's solution.

2. **Ammoniacal Silver Test.**—Warm picrotoxin with aqueous silver nitrate solution containing a slight excess of ammonium hydroxide solution. The reducing action of picrotoxin will produce a black precipitate of metallic silver, or a dark brown color when only traces are present.

3. **Oxidation Test.**—Picrotoxin, treated with a little concentrated sulphuric acid in a porcelain dish, first becomes orange-red and then dissolves when stirred forming a reddish yellow solution. A drop of potassium dichromate solution will produce a red-brown color around the margin of the drop. If the two liquids are thoroughly mixed, an immediate dirty brown color appears, passing into green on long standing.

A green color alone is without significance, since many organic substances capable of reducing chromic acid to chromic oxide produce the same result.

4. **Melzer's Test.**[1]—Put some picrotoxin upon a watch-glass and add 1–2 drops of a mixture of benzaldehyde and absolute alcohol. Careful addition of a drop of concentrated sulphuric acid will produce a distinct red color. If the watch-glass is tilted back and forth, red streaks will run from the substance through the liquid.

Use a freshly prepared 20 per cent. solution of benzaldehyde in absolute alcohol. Benzaldehyde alone gives a yellowish brown color with concentrated sulphuric acid. Alcohol is added as a diluent to diminish this color as much as possible. Under these conditions the solution has a light yellow color, and the dark red tint caused by picrotoxin is very clearly defined. This red color is unstable and, beginning at the margin, gradually fades into a pale pink or violet. Kreis[2] has found that cholesterol and phytosterol[3] give similar colors with Melzer's reagent. Veratrine gives a red color like that with sulphuric acid alone. Morphine gives fine red to yellow-red streaks or colors.

5. **Langley's Test.**—Mix picrotoxin with about 3 times the quantity of potassium nitrate and moisten the mixture with the smallest

[1] H. Melzer: Contributions to Forensic Chemistry. Zeitschr. f. analyt. Chem. 37 (1898), 351 and 747.

[2] H. Kreis: Contribution to the Knowledge of Melzer's Picrotoxin Test. Chemiker-Zeitung 23 (1899), 21.

[3] A substance very similar to cholesterol, and named paracholesterol or phytosterol, is found in the seeds of certain plants. (Perkin and Kipping, Organic Chemistry, page 574.)

possible quantity of concentrated sulphuric acid. Then add strong sodium hydroxide solution in excess and an intense red color will appear.

6. Physiological Test.—The stupefying, intoxicating action of picrotoxin upon fish may be used for its detection. This test may be made by dissolving the substance to be examined for picrotoxin, depending upon its quantity, in 250–500 cc. of spring or tap-water and introducing a small fish weighing about 1 gram. After some time fish reach a condition of great irritation, sometimes turning on their long axis, losing the power of swimming in the normal position, and lying on their sides. One milligram of picrotoxin is said to kill rather small fish in 7 hours and 9.5–10 milligrams in 2.5 hours. The haemolytic action of picrotoxin in physiological salt solution may also be used for its detection; likewise its action in causing convulsions. Picrotoxin is a convulsive poison, unlike strychine not producing extension of the hind legs. Fühner[1] has made the following observations: If 0.2 mg. of picrotoxin is injected into a medium-sized frog, the animal after several hours will no longer rest upon its front legs; it keeps its hind legs mostly in a bent position; and moreover there is tension of the webs. Even after 24 hours there is stiffness and an inclination to take this characteristic "picrotoxin position." In the case of 0.5 mg. the first convulsion occurs after about 45 minutes. The animals may be thrown upon their backs and then into the abdominal position. These convulsions may persist for several hours. With 1 milligram, convulsions that begin to be tetanic in character, appear after about 1 hour. Usually severe convulsions occur later spontaneously, or frequently better after irritation in conjunction with full inflation of the thorax and its sudden evacuation with a sharp cry. These convulsions with and without cry may be repeated for several hours. They gradually become weaker and finally the animal dies completely paralyzed.

Detection of Picrotoxin in Beer and Urine

Start with at least 2 liters of beer. If it has a faint acid reaction, make it neutral with magnesium oxide and evaporate upon the water-bath to a thin syrup. Digest this residue with 5 times its volume of alcohol, evaporate the filtered alcoholic extract, dissolve the residue in hot water, filter through a moistened paper, acidify the filtrate with dilute sulphuric acid, and extract repeatedly with ether, or better with chloroform. Should the residue from ether or chloroform be too impure, dissolve again in hot water, filter, evaporate, and extract with ether or chloroform. To purify picrotoxin further, precipitate colored substances from its aqueous solution with lead acetate, filter and remove lead from the filtrate by hydrogen sulphide. The filtrate from lead sulphide upon evaporation, or extraction with ether or chloroform, will give nearly pure picrotoxin. The very bitter taste of picrotoxin, its strong tendency to crystallize from a little hot water, the chemical reactions given above, and especially the physiological experiment with a small, lively fish, serve for its identification.

The detection of picrotoxin in any other material, such as urine, may be undertaken in the manner described above.

[1] H. Fühner: Detection and Estimation of Poisons Biologically, Abderhalden's Biochemische Arbeitsmethoden, Vol. V, Part I, Page 46 (1911).

COLCHICIN

Colchicin, $C_{22}H_{25}NO_6$, an alkaloid occurring in all parts of the meadow saffron, Colchicum autumnale, is found most abundantly in the ripe seeds, especially in the hulls (0.3–0.8 per cent.) and in the bulbous buds collected before they bloom (0.2–0.5 per cent.). It occurs in smaller quantity in the fresh flowers (0.01–0.02 per cent.) and in the fresh leaves. According to the statements of other investigators, the fresh flowers contain 0.1–0.6 per cent. of colchicin.

Colchicin is a yellowish, amorphous powder, highly poisonous and very bitter to the taste. It is freely soluble in water, alcohol, and chloroform, less so in ether and benzene, and almost insoluble in petroleum ether. The yellowish solutions of colchicin possess only very weak basic properties. Consequently ether or chloroform, but not benzene or petroleum ether, will extract colchicin from an aqueous, tartaric acid solution. Upon evaporation of the solvent, colchicin will appear as a yellowish, sticky residue resembling a resin or varnish. Extraction of colchicin is more complete, if the aqueous solution is made alkaline with ammonia and extracted with chloroform. Dry colchicin upon exposure to light becomes darker in color.

Constitution of Colchicin.—When boiled with acidulated water, colchicin undergoes cleavage into methyl alcohol and colchiceïn. The latter is a weak acid, containing according to Zeisel a free carboxyl-group, colchicin being the methyl ester of this acid:

$$\underset{\text{Colchicin}}{C_{20}H_{22}O_4NCOOCH_3} + H_2O = \underset{\text{Colchiceïn}}{C_{20}H_{22}O_4NCOOH} + CH_3.OH.$$

Positive proof of the presence of a carboxyl-group in the colchiceïn molecule has not yet been furnished. According to A. Windaus, the possibility that colchiceïn is an enol-form must be taken into consideration and that colchicin is the corresponding methyl ether of this enol-form:

$$\underset{\text{Colchicin}}{C_{21}H_{22}O_5N(OCH_3)} + H_2O = \underset{\text{Colchiceïn}}{C_{21}H_{22}O_5N(OH)} + CH_3.OH.$$

This view of Windaus finds support in the fact that colchicein, but not colchicin, gives a green coloration with ferric chloride. These color-reactions with ferric chloride are characteristic of phenols and enol-forms but not of carboxylic acids.

On the other hand, colchicin is formed when colchiceïn is heated at 100° with sodium methylate and methyl iodide.

Since colchiceïn upon treatment with hydriodic acid yields three molecules of methyl iodide, colchiceïn as well as colchicin contains three methoxyl-groups. Heated with strong hydrochloric acid, colchiceïn loses acetic acid and passes into trimethyl-colchicinic acid. The latter is capable of forming salts with

bases and also with acids. Zeisel assumes that in the formation of this acid an acetyl-group attached to nitrogen is split off:

$$\underset{\text{Colchicein}}{C_{19}H_{18}O_4(OH)(NH.CO.CH_3)} + H_2O = \underset{\text{Trimethyl-colchicinic acid}}{C_{19}H_{18}O_4(OH)(NH_2)} + CH_3.COOH.$$

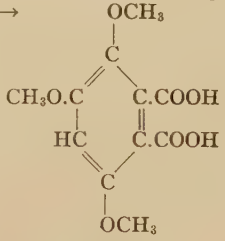
According to Zeisel, trimethyl-colchicinic acid still contains three methoxyl-groups. They are attached to a benzene ring, for by the oxidation of this acid Windaus has obtained 3, 4, 6-trimethoxy-1, 2-phthalic acid. For the cleavage-products isolated by Zeisel and Windaus[1] and for colchicin itself the following formulae have been proposed:

Colchicin, $C_{22}H_{25}O_6N \rightarrow (CH_3O)_3C_6H:(C_{10}H_8O)$ with OCH_3 and $NH.CO.C.\ H_3$

Colchicein, $C_{21}H_{23}O_6N \rightarrow (CH_3O)_3C_6H:(C_{10}H_8O)$ with OH and $NH.CO.CH_3$.

Trimethyl-colchicinic acid, $C_{19}H_{21}O_5N \rightarrow (CH_3O)_3C_6H:(C_{10}H_8O)$ with OH and NH_2.

3, 4, 6-Trimethoxy-1, 2-phthalic acid, $C_{11}H_{22}O_7 \rightarrow$



Toxic Action.—Absorption of colchicin takes place very slowly. The symptoms of poisoning are: emesis and cholera-like stools, pains in the back, arms and legs, spasms and convulsions, dyspnoea, and disturbances of vision and speech. The body-temperature may drop abnormally, so that the skin feels cold. There is intense thirst and nausea, also micturition, and albuminuria may appear. Yet the urine may also be normal but always deficient in quantity. In consequence of poor absorption of colchicin, symptoms of intoxication usually do not appear for several hours, and its strong cumulative action may be attributed to this cause. Colchicin belongs without doubt among the most potent poisons and a single dose of 3 mg. has been instrumental in bringing about the death of a man.

Detection of Colchicin

Aqueous colchicin solutions, especially in presence of dilute mineral acids, have a yellow color. Consequently if a few drops of

[1] A. Windaus: Investigations upon Colchicin. I and II Sitzungsber. d. Heidelberger Akad. d. Wiss. Jg. 1910 and 1911.

dilute hydrochloric or sulphuric acid are added to a nearly colorles colchicin solution, it becomes more or less intensely yellow. Unles the ether residue has this characteristic, colchicin is absent.

Colchicin and colchiceïn give the same reactions.

1. **Tannic Acid Test.**—This reagent will precipitate colchicin from aqueous solution, if not too dilute, as white flocks. This test, however, is not characteristic of colchicin.

2. **Nitric Acid Test.**—Nitric acid (sp. gr. 1.4 = 66 per cent.) dissolves colchicin with a dirty violet color that soon changes when stirred to brownish red and finally to yellow. Addition of dilute sodium or potassium hydroxide solution, until the reaction is alkaline, produces an orange-yellow or orange-red color.

3. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves colchicin with an intense yellow color. A drop of nitric acid, or a particle of potassium nitrate, added to such a solution produces a green, blue, violet and finally a pale yellow tone. Excess of potassium hydroxide solution will now develop an orange-red color. Erdmann's and Froehde's reagents dissolve colchicin with an evanescent blue to violet color soon passing into yellow.

4. **Hydrochloric Acid Test.**—Concentrated hydrochloric acid dissolves colchicin with an intense yellow color. Add 2–6 drops of ferric chloride solution and heat the mixture 2–3 minutes in a test-tube until half the liquid has evaporated. A deep olive-green color appears, becoming darker on cooling and rendered permanent by dilution with water. Finally shake the solution with a few drops of chloroform. The latter becomes yellowish brown, or garnet-red, the aqueous solution retaining its green color. This test given by Zeisel is due to saponification of methoxyl-groups, formation of phenols, and reaction of the latter with ferric chloride.

Führer's[1] Procedure for Zeisel's Test

Führer recommends a combination of chemical and biological tests for the forensic detection of colchicin. In Zeisel's test, it is best to bring about preliminary cleavage of colchicin for the color reaction by warming with about 5 drops of 20 per cent. hydrochloric acid, because if hydrolytic cleavage of colchicin is carried beyond the colchiceïn stage, ferric chloride does not produce a red product

[1] H. Führer: Toxicological Detection of Colchicin. Arch. f. exper. Path. u. Pharm. 63 (1910), 357.

soluble in chloroform. According to Führer, white mice may be used in the biological detection of minute quantities of colchicin. After 24 hours they die with severe diarrhoea from 0.1 mg. of colchicin-Boehringer. Immunity of frogs to colchicin, as to tetanus toxin, may be destroyed by warmth. At 30–32° a frog will die in 2–4 days from 0.1 mg. of colchicin-Boehringer. Toxicity of colchicin for frogs at this temperature, as compared with room temperature, is 500 times greater, so that frogs also may be used in the biological detection of colchicin. Colchicin administered subcutaneously is partly eliminated by frogs in an active form in urine. In isolating colchicin from the organism according to Führer, the gastro-intestinal contents together with the faeces of the poisoned animal are extracted with alcohol. The residue from the extract in aqueous solution is extracted with petroleum ether to remove fat and then with chloroform. The residue from this chloroform solution is tested biologically.

Material for Examination.—The best material to use for the detection of colchicin in suspected poisoning is first vomitus, gastric and intestinal contents, and faeces; and then blood, kidneys and urine. Colchicin resists putrefaction for 3–6 months, since it is said to act as a preservative.

Baumert (see Ptomaines, page 457) isolated a cadaveric colchicin from a 22 months old cadaver by extracting the aqueous tartaric acid solution with ether. Like colchicin it dissolved very readily in chloroform. The yellow, amorphous residue had a distinctly bitter as well as sharp taste. Concentrated sulphuric, dilute hydrochloric and nitric acids, dissolved it with yellow color. Toward concentrated nitric acid its behavior was like that of colchicin. Unlike colchicin, however, this ptomaine did not give Zeisel's test.

Purification of Colchicin Residue from Ether or Chloroform Extract

To isolate colchicin as pure as possible from the yellow residue, extract with warm water. Filter the solution and extract when cold first with petroleum ether. This will remove fatty, resinous and colored impurities but not colchicin. Then extract with chloroform. Or precipitate colchicin from not too dilute aqueous solution, with tannic acid. Collect this precipitate upon a filter and wash with cold water. Mix the moist precipitate with freshly precipitated, washed lead hydroxide. Dry the mixture, grind to a powder and extract with chloroform. Evaporation of the solvent will leave nearly pure colchicin as a yellow, varnish-like mass that will give the colchicin reactions, especially Zeisel's test, very well.

PICRIC ACID

Picric acid is 2,4,6-trinitro-phenol, $C_6H_2(NO_2)_3(OH)$ and is formed from many benzene derivatives by nitric acid, for example, from aniline, salicylic acid, salicine, indigo, phloridzine; also from such materials as silk, wool and many resins, particularly aloes and gum benzoin.

Preparation.—Phenol and concentrated sulphuric acid are heated until a test portion gives a clear solution in a little water. The phenol-sulphonic acid formed (α) is dissolved in water and, while the solution is stirred, nitric acid (sp. gr. 1.4 = 61 per cent.) is gradually introduced (β). Vigorous nitration begins with copious evolution of red fumes of nitrogen peroxide and is finally brought to completion by warming the mixture upon the water-bath. The mass which becomes crystalline upon cooling is freed from admixed nitric-sulphuric acid in a press or centrifuge and washed with cold water. It is then recrystallized from hot water. Picric acid crystals should not be dried at a temperature above 40°, otherwise decomposition may take place with explosive violence.

(α) $HO.C_6H_4.H + HO.SO_2.OH = H_2O + HO.C_6H_4.SO_2.OH$,

(β) $HO.C_6H_4.SO_2.OH + 3HNO_3 = HO.C_6H_4(NO_2)_3 + 2H_2O + H_2SO_4$.

Properties.—Picric acid crystallizes from water in light yellow, shining leaflets and from ether in lemon-yellow prisms. It dissolves in 86 parts of water at 15°, forming an acid solution having a strong yellow color and intensely bitter taste. It is soluble in 9 parts of alcohol and in 44 parts of ether. The ether solution is only slightly colored and the chloroform and petroleum ether solutions scarcely at all. This behavior is attributed to the fact that picric acid is scarcely ionized in the two last-mentioned solvents. It melts at 122° and may be sublimed when carefully heated. Picric acid can be titrated with n-potassium hydroxide solution with phenol-phthalein as indicator. In titration it behaves like a strong monobasic acid. Solutions of picric acid act as a direct, permanent yellow dye for such animal materials as wool, silk and skin, but not for materials of vegetable origin, such as cotton and paper. Salts of picric acid, called picrates, usually crystallize well. With salts of many alkaloids, picric acid forms yellow, crystalline precipitates of alkaloidal picrates.

Toxic Action and Elimination.—Picric acid is quite a vigorous poison. Taken internally it produces a striking yellow pigmentation first of the conjunctiva and then of the entire skin, usually designated as "picric acid icterus." Picric acid and its salts like most nitro-compounds decompose red blood-corpuscles forming methaemoglobin. Consequently it is a blood-poison. It is further a convulsive poison, for it irritates the central nervous system causing convulsions. Finally it exercises its power of precipitating proteins in acid solution. This manifests itself by necrotic tissue changes especially in those organs of the body that have an acid reaction, such as the stomach and kidneys. The organism reduces picric to picramic acid, $C_6H_2(NO_2)_2(NH_2)(OH)$, which does not so readily precipitate protein. By thus changing picric acid the organism rids

itself of the poison. In picric acid poisoning the urine has a marked red color owing to formation of picramic acid. Some picric acid passes into the urine unchanged. Elimination is slow. In one case (see R. Kobert "Intoxikationen"), after administration of a single dose of 1 gram of picric acid, its presence in the urine could be recognized for 6 days. The urine was ruby-red, clear, acid, and free from albumin and bile-constituents. Picric acid was also easily detected in the faeces.

Detection of Picric Acid

Material containing picric acid has a more or less yellow or yellowish green color. Aqueous, alcoholic and ethereal solutions have the same color. Finely divided animal material should be extracted several hours under a reflux with alcohol containing hydrochloric acid to decompose compounds of picric acid with albumins and thus bring the acid into solution. Filter and evaporate such an alcoholic extract upon the water-bath. Treat the yellow, yellowish green, or frequently yellowish red or reddish brown residue with warm water and filter the extract. Test the filtrate itself direct for picric acid, or first extract as usual with considerable ether. Tests may then be applied to the residue left by evaporating the ether extract. Picric acid is easily extracted by ether or chloroform from a solution strongly acid with mineral acid but not from one faintly acid. This behavior is in full accord with its slight ionization in ether and chloroform. Consequently only the un-ionized portions, not the ions, of picric acid are susceptible of extraction by these solvents. Addition of hydrochloric or sulphuric acid diminishes, that is, strongly represses ionization of picric acid, thus increasing the ease with which it can be extracted.

Reactions of Picric Acid

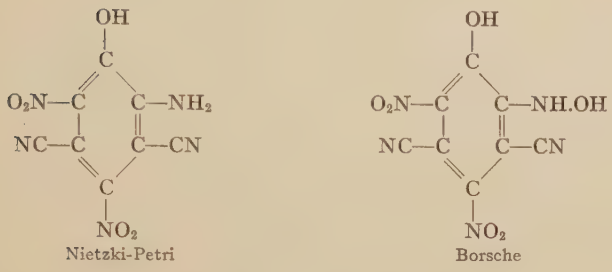
1. **Isopurpuric Acid Test.**—Gently heat (50–60°) an aqueous solution of picric acid with a few drops of saturated aqueous potassium cyanide solution (1:2). The solution will become deep red owing to formation of potassium isopurpurate. Even one mg. of picric acid dissolved in 5 cc. of water (1:5000) gives a deep red color.

Isopurpuric acid does not exist in the free state but is present in this test as the potassium salt. Nietzki and Petri[1] regard isopurpuric acid as a dicyanopicramic acid = 5-oxy-6-amino-2,4-dinitro-isophthalic nitrile, whereas Borsche[2] considers it a dicyano-dinitro-oxy-β-phenyl-hydroxylamine:

[1] R. Nietzki and W. Petri: Constitution of Isopurpuric Acid. Ber. d. Deutsch. chem. Ges. 33 (1900), 1788.

[2] W. Borsche: Constitution of Meta-purpuric Acid. Ber. d. Deutsch. Chem. Ges. 33 (1900), 2995.

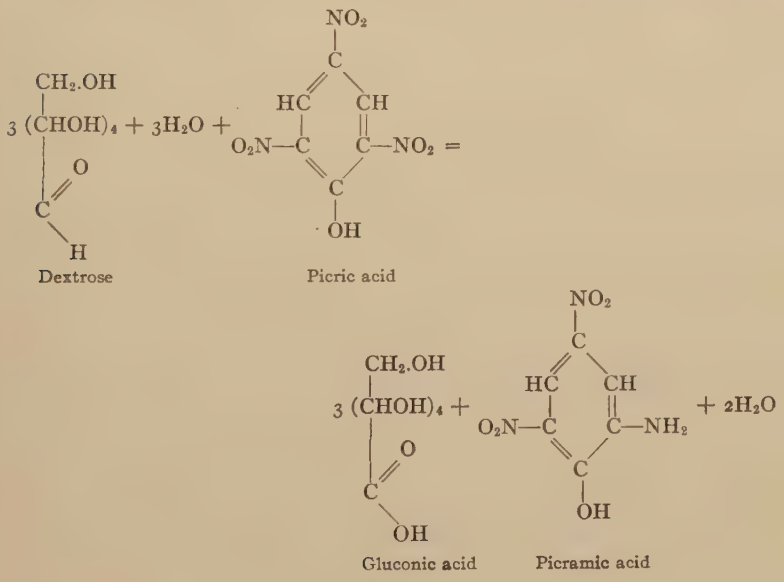
Structural Formulæ of Isopurpuric Acid



2. **Picramic Acid Test.** (a) **With Dextrose.**—Heat an aqueous picric acid solution with 2–3 drops each of aqueous sodium hydroxide and dextrose solution. The solution becomes dark red. Avoid excess of sodium hydroxide solution, otherwise a red color due solely to the action of the alkali upon dextrose will appear.

(b) **With Ammonium Sulphide.**—The same red color appears when a picric acid solution is warmed with a few drops of sodium hydroxide and ammonium sulphide solution.

In both cases picric acid is reduced to picramic acid, 2-amino-4-6-dinitro-phenol. The following equation explains formation of the first oxidation product, gluconic acid, in the test with dextrose:



3. **Ammoniacal Copper Test.**—Add a few drops of ammoniacal copper sulphate solution to an aqueous picric acid solution. A yellowish green precipitate, consisting of hexagonal needles having a polarizing action upon light, will appear. A solution of even 1 mg. of picric acid in 8 cc. of water gives such a precipitate.

4. **Dyeing Test.**—Dissolve the substance containing picric acid in hot water and put threads of wool, silk and cotton in the solution. In a few hours (12–24) remove the threads and thoroughly rinse in pure water. If picric acid is present, the wool and silk will be dyed yellow but not the cotton. In other words, picric acid is not fast upon vegetable fibers like cotton. Picric acid diluted 1:100,000 will still produce a yellow color upon wool.

ACETANILIDE

Acetanilide, or antifebrine, is acetylated aniline having the composition $C_6H_5.NH.CO.CH_3$.

Preparation.—Boil aniline and glacial acetic acid together for several hours under a reflux, until a test portion hardens when cooled and stirred. Acetanilide thus formed is recrystallized from hot water:

$$C_6H_5.NH_2 + CH_3.COOH = H_2O + C_6H_5.NH.CO.CH_3.$$

Acetanilide crystallizes from water in colorless shining leaflets melting at 113–114° and having a faint burning taste. It is soluble in 230 parts of cold water, in about 22 parts of boiling water, and in 3.5 parts of alcohol. It is freely soluble in ether and still more so in chloroform. All acetanilide solutions are neutral. Heated to boiling with potassium hydroxide solution (I) and also with fuming hydrochloric acid (II), acetanilide undergoes hydrolytic cleavage into its constituents:

I. $C_6H_5.NH.CO.CH_3 + KOH = C_6H_5.NH_2 + CH_3.COOK.$

II. $C_6H_5.NH.CO.CH_3 + HCl + H_2O = C_6H_5.NH_2.HCl + CH_3.COOH.$

Ether or chloroform will completely extract acetanilide from an aqueous acid solution.

Toxic Action.—As a derivative of aniline, acetanilide possesses the poisonous properties of that amine, though in less degree by reason of the presence of the acetyl-group. Several cases are on record where severe symptoms of intoxication appeared from taking rather large quantities of acetanilide. As yet, however, poisonings with fatal termination have not occurred. If acetanilide were more soluble in water and in the body-fluids, amounts of 28 grams which have been taken at one time would probably have proved fatal. R. Kobert has reported a case where a student took a teaspoonful of the drug. There was stupor, uneasiness, marked cyanosis, and lowering of pulse. A purgative and restorative (stimulant) were used but there was considerable exhaustion for several days. A man who took 2 grams of antifebrine daily for 2 days in succession gave nearly the same picture. In the case of a man 24 years old, who

had taken 12 grams of antifebrine with suicidal intent, there appeared: after half an hour dizziness, swimming of objects before the eyes, nausea, increase in acidity, burning in the stomach, cyanosis and syncope. Cyanosis persisted 2 days later, in spite of lavage of the stomach and injection of camphor. Neither albumin nor methaemoglobin was eliminated in the urine. This is the more remarkable, since aniline itself is a typical methaemoglobin-former. In isolated cases a red skin eruption was observed.

Detection of Acetanilide

1. Indophenol Test.—This test is due to presence of a free, primary, aromatic amino-group (NH_2). Therefore acetanilide must first be hydrolyzed.

Procedure.—Boil acetanilide in a small test-tube with about 4 cc. of fuming hydrochloric acid and evaporate the solution to about 10 drops. Cool and add 2–4 cc. of saturated, aqueous carbolic acid solution. Freshly prepared calcium hypochlorite solution, added drop by drop with good shaking, will produce a more or less intense dirty red-violet color. The color will become deeper, if the mixture is well-shaken for a few minutes. Then carefully add ammonium hydroxide solution as an upper layer. The latter will become a beautiful indigo-blue, whereas the under layer will retain a red color. This blue color is only characteristic of acetanilide and other aniline derivatives when preceded by the dirty red-violet color, since a mixture of an aqueous phenol solution and hypochlorite solution gives a blue color with ammonia. Phenacetine also gives the indophenol test.

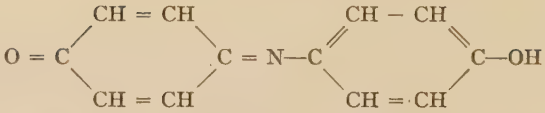
Explanation.—Aniline (see above) produced from acetanilide by hydrolytic cleavage is oxidized by calcium hypochlorite to p-amino-phenol:

$$C_6H_5.NH_2 + O = HO.C_6H_4.NH_2.$$

p-Amino-phenol then reacts with phenol, also under the influence of oxidation, forming with loss of 4 atoms of hydrogen indophenol:

$$\begin{array}{r} +O+O \\ H\boxed{O.C_6H_4.N}\boxed{H_2}+H \end{array} C_6H_4.OH = 2H_2O + N\begin{array}{l} \diagup\!\!=C_6H_4=O \\ \diagdown\ C_6H_4.OH \end{array}$$

Therefore, in order that an indophenol dyestuff may be formed, only those primary, aromatic amines are available in which there is either an open para-position to the amino-group, or groups in that position that can easily be removed under the influence of oxidation. Such is the case with phenacetine, whereas anaesthesine, $H_2N.C_6H_4.COOC_2H_5(1,4)$, does not give the indophenol test. Indophenol is soluble in alcohol with a red color and in sodium hydroxide solution, or ammonia, with a blue color. Its structural formula is as follows:



2. **Phenyl-isocyanide Test.**—Boil acetanilide for a few minutes with about 5 cc. of alcoholic or aqueous potassium hydroxide solution. Cool, add 2 drops of chloroform and heat again. The offensive odor of phenyl-isocyanide will be developed. Potassium hydroxide hydrolyzes acetanilide, forming potassium acetate and aniline (see above), and the latter with chloroform gives phenyl-isocyanide (see Chloroform, page 60). Phenacetine and other derivatives of p-phenetidine do not give the phenyl-isocyanide test.

3. **Calcium Hypochlorite Test.**—Boil acetanilide for a few minutes with alcoholic potassium hydroxide solution. Dilute with water and extract aniline with ether. Remove the ether extract, evaporate upon a watch-glass, and test the residue for aniline by means of calcium hypochlorite solution or by the phenyl-isocyanide test.

4. **Azo-dyestuff Test.**—The reactions involved in this test require the presence of a free, primary amino-group. Acyl-derivatives of primary aromatic bases, such as acetanilide or phenacetine, must first be hydrolyzed by boiling with concentrated hydrochloric acid (see above).

Procedure.—Boil acetanilide, as described in the indophenol test, for a few minutes with fuming hydrochloric acid. Dilute with 3–5 cc. of water, cool well (best in ice), add a few drops of potassium nitrite solution (1:10), render alkaline while cooling with sodium hydroxide solution, and add carefully as an upper layer an alkaline solution of β-naphthol. A red zone will appear at the contact-surface of the two liquids. Different shades of red are given by the different primary aromatic bases.

Explanation.—Nitrous acid converts the hydrochloride of the primary base into a diazonium chloride which sodium hydroxide solution changes to the corresponding diazonium hydroxide. In alkaline solution the latter will couple with a phenol, such as β-naphthol, forming an oxyazo-dyestuff:

(α) $C_6H_5.NH_2.HCl + HO.NO = 2H_2O + C_6H_5.\overset{\cdot\cdot}{N}.Cl$ Diazo-benzene chloride.

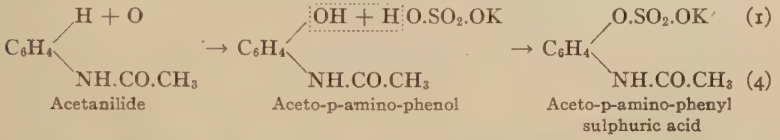
(β) $C_6H_5.N_2.Cl + NaOH = NaCl + C_6H_5.N = N.OH$ Diazo-benzene hydroxide.

(γ) $C_6H_5.N = N.OH + H.C_{10}H_6.OH = H_2O + C_6H_5.N = N.C_{10}H_6.OH$ β-Naphthol-oxyazobenzene.

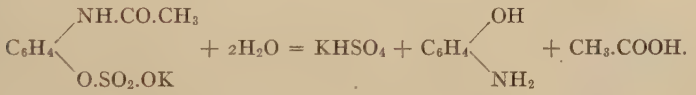
5. Detection of Acetyl Group.—When the amount of material is not too small, the test for acetic ether may be made. Heat for a few minutes in a boiling water-bath, mixed in a small test-tube with concentrated sulphuric acid and some alcohol. If acetanilide is present, the characteristic odor of ethyl acetate will appear upon careful addition of a little water.

Examination of Acetanilide Urine[1]

Human urine contains acetanilide as such only after rather large doses have been taken. Most of the acetanilide is oxidized in the human organism to aceto-p-amino-phenol, which like nearly all phenols undergoes conjugation with acid potassium sulphate forming an ethereal sulphuric acid, aceto-p-amino-phenyl-sulphuric acid which is eliminated in the urine:



Part of the aceto-p-amino-phenol undergoes conjugation in the human body with glycuronic acid. These conjugated acids, heated with concentrated hydrochloric acid, are hydrolyzed. One component of the cleavage, p-amino-phenol, gives the indophenol test:



An "acetanilide urine," boiled for a few minutes with concentrated hydrochloric acid, usually gives the indophenol test. But the test is more certain, if p-amino-phenol is first isolated. Boil a considerable quantity of urine (300–500 cc.) for a few minutes with about 10 cc. of concentrated hydrochloric acid under a reflux. Then add excess of sodium carbonate and repeatedly extract the cold urine with large quantities of ether. Distil or evaporate the ether. The residue usually contains p-amino-phenol as a reddish or brownish oil. An aqueous solution of this substance will give the indophenol test.

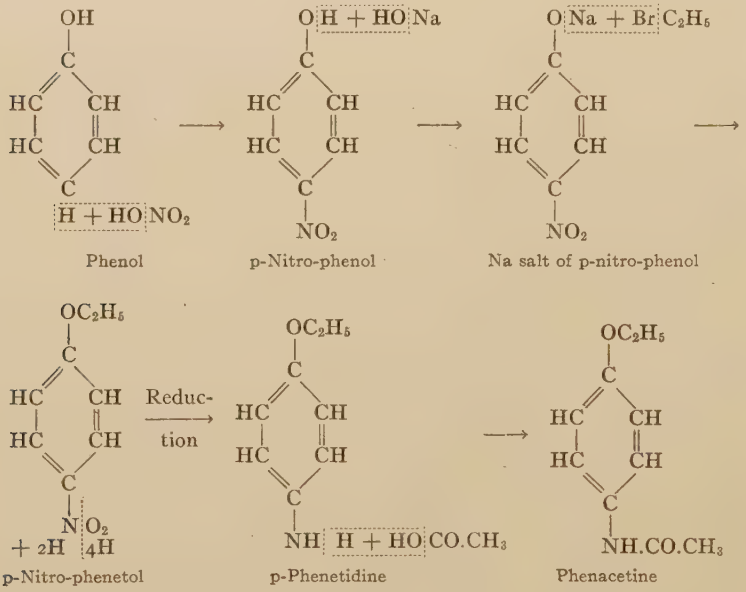
PHENACETINE

Phenacetine, p-aceto-phenetidine, $C_2H_5O.C_6H_4.NH.CO.CH_3$ (1, 4), crystallizes in shining leaflets without color, odor or taste, and melts at 134–135°. It is soluble in 1400 parts of cold water, in about 70

[1] To study the behavior of acetanilide in the human organism, take at night twice in the course of 3 hours 0.3 gram of this compound at a dose and examine in the manner described above the urine passed in the next 12 hours.

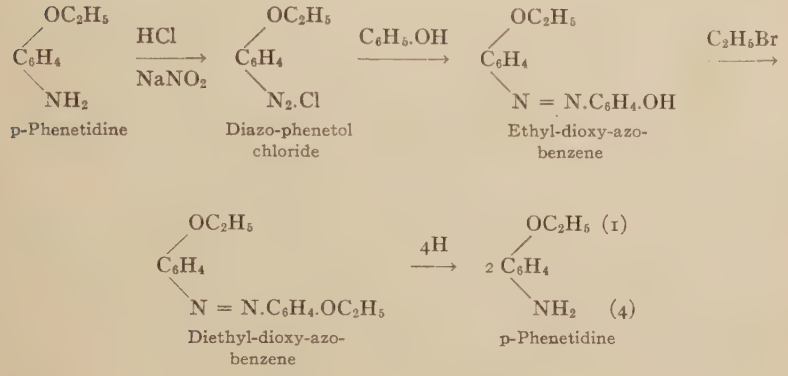
parts of boiling water, 16 parts of alcohol, and freely soluble in ether and chloroform. Its solutions are neutral. Concentrated sulphuric acid dissolves it without color. Phenacetine can be easily and completely extracted by ether or chloroform from an acid, aqueous solution.

Preparation.—Gradual addition of crystallized phenol to cold dilute nitric acid (sp. gr. 1.11 = 17.5 per cent.) results in the formation of a mixture of o- and p-nitro-phenol. Since the o-compound is volatile with steam, complete separation of the two products is possible by steam distillation. Residual p-nitro-phenol is converted into its sodium salt by sodium hydroxide solution and then into p-nitro-phenetol by ethyl bromide. This product is reduced by nascent hydrogen from tin and hydrochloric acid to p-amino-phenetol = p-phenetidine, which is finally boiled with glacial acetic acid and converted into aceto-p-phenetidine = phenacetine:



According to Riedel, p-phenetidine is diazotized in hydrochloric acid solution by sodium nitrite and converted into diazo-phenetol chloride. The latter coupled in alkaline solution with phenol gives ethyl-dioxy-azo-benzene which upon ethylation by ethyl bromide and sodium hydroxide solution yields diethyl-dioxy-azo-benzene. This compound reduced by nascent hydrogen from tin and hydrochloric acid gives two molecules of p-phenetidine. Half of this final product is boiled for several hours with glacial acetic acid and converted into

phenacetine, whereas the other half is passed again through the reactions just described:



Physiological Action.—In man phenacetine does not bring about decomposition of blood with formation of methaemoglobin, when the dose required as a febrifuge or antineuralgic is not exceeded. In one instance Krönig observed occurrence of death accompanied by very severe decomposition of blood in the case of a 16 year old compositor who had taken 1 gram of phenacetine. According to Lewin, phenacetine may give rise to cyanosis with and without collapse. A woman had taken 2 grams of phenacetine in 3 hours. Although the symptoms that appeared after the first gram were mainly nervous, such as dizziness and trembling, after the second gram taken 3 hours later there was a feeling of intense cold, severe cyanosis of the fingers, hands, lips and cheeks, deep blue coloration of the skin with cold sweat, feeling of anxiety and dyspnoea. This coloration still persisted four hours later. Even the palate and mucous surfaces were cyanotic. Not until 12 hours had elapsed did this condition begin to abate. In this as in every case where blood decomposition occurs, notwithstanding the blue coloration due to methaemoglobin, icterus could be detected locally. As often happens in methaemoglobinaemia, the blood in phenacetine intoxication gives evidence of changes in red blood-corpuscles from which the pigment has either wholly disappeared or has agglomerated in one place. The secondary effects upon the skin also deserve mention. Exanthema of the skin may occur, chiefly in the extremities, in persons with and without fever. Red spots that coalesce and give rise to burning may appear and fade out on the following day. Swellings of the skin may also occur. After a dose of 2 grams of phenacetine, taken at one time in the evening, there was observed in the case of a man suffering from ischias an oedematous swelling of both the lower eyelids. After further administration of 3 grams in two doses, the same kind of swelling appeared on the forehead, the bridge of the nose, and the upper part of the cheeks. Moreover all the fingers were swollen to such an extent that they could not be bent at the joints (Lewin).

Detection of Phenacetine

As a substituted acid-amide, phenacetine undergoes hydrolytic cleavage into p-phenetidine and acetic acid, when heated with potassium hydroxide solution or concentrated hydrochloric acid:

$$C_2H_5.O.C_6H_4.NH.CO.CH_3 + KOH = C_2H_5.O.C_6H_4.NH_2 + CH_3COOK.$$

1. **Oxidation Test.**—Boil phenacetine for several minutes with about 3 cc. of concentrated hydrochloric acid. Dilute with water to about 10 cc. and filter when cold. Addition to the filtrate of a few drops of 3 per cent. chromic acid solution will produce a ruby-red color. In this test acetanilide gives a yellow color gradually turning green. Strong chlorine water may be substituted for chromic acid.

2. **Indophenol Test.**—Treated in the manner described for acetanilide (see page 123), phenacetine gives a very good indophenol test.

3. **Azo-dyestuff Test.**—After preliminary boiling with concentrated hydrochloric acid, phenacetine gives a distinct test due to formation of an azo-dyestuff, as described under acetanilide (see page 124).

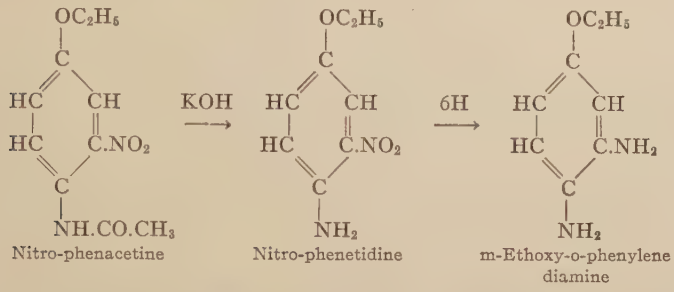
4. **Autenrieth-Hinsberg Test.**[1] (a) **With Dilute Nitric Acid.**—Heat phenacetine to boiling with dilute nitric acid (10–12 per cent.). It dissolves with a yellow to orange-red color. If the solution is saturated, it will deposit upon cooling long, yellow needles of mono-nitro-phenacetine, $C_6H_3(NO_2)(OC_2H_5)(NH.CO.CH_3)$, melting at 103°. This test is delicate and characteristic of phenacetine, when nitro-phenacetine can be obtained in crystals and sufficiently pure for a determination of melting-point. Acetanilide and antipyrine give colorless solutions when warmed with dilute nitric acid.

(b) **With Concentrated Nitric Acid.**—A few drops of concentrated nitric acid added to phenacetine produce a yellow to orange color. Part of the phenacetine is dissolved with the same color. Warming, especially upon addition of water, dissolves it completely and nitro-phenacetine crystallizes in yellow needles as the solution cools.

When phenacetine is nitrated, the nitro-group takes the position ortho to the acetamino-group. This is shown by the fact that nitro-phenacetine, when heated with alcoholic potassium hydroxide solution, gives nitro-phenetidine crystallizing in ruby-red needles. The latter upon reduction with nascent

[1] W. Autenrieth and O. Hinsberg: Contribution to the Knowledge of Phenacetine and m-Ethoxy-o-Phenylene-Diamine. Arch. d. Pharm. 229 (1891), 456.

hydrogen from zinc dust and potassium hydroxide solution is converted into an ortho-diamine:



Nitro-phenacetine — Nitro-phenetidine — m-Ethoxy-o-phenylene diamine

5. **Differentiation Test.**—Phenacetine differs from acetanilide in not giving the phenylisocyanide test, when warmed with chloroform in presence of potassium hydroxide solution.

Phenacetine Urine

After rather large doses, phenacetine sometimes appears in the urine unchanged. Used in moderate doses, phenacetine undergoes hydrolytic cleavage in the human body with formation of p-phenetidine and p-amino-phenol. These compounds are eliminated in the urine either by themselves or as conjugates of sulphuric and glycuronic acid:

$$CH_3.CO.NH.C_6H_4OC_2H_5 \xrightarrow{\text{hydrolysis}} H_2N.C_6H_4.OC_2H_5 \text{ and } H_2N.C_6H_4.OH,$$

$$H_2N.C_6H_4.OH + H.O.SO_2.OK \xrightarrow{\text{conjugation}} H_2N.C_6H_4.O.SO_2.OK.$$

Therefore phenacetine-urine usually gives the indophenol and azo-dye test. These tests are always given, if the urine has previously been boiled with hydrochloric acid.

Procedure.—Heat 10–20 cc. of urine to boiling with a few drops of concentrated hydrochloric acid. Cool (best with ice), add 2–3 drops of 10 per cent. sodium nitrite solution, shake, then add 2 drops of 5 per cent. alcoholic α-naphthol solution, and finally render alkaline with sodium hydroxide solution. If p-phenetidine or p-amino-phenol is present, a red color will appear and change to a beautiful dark cherry-red upon addition of hydrochloric acid. Edlefsen[1] prefers the alcoholic to the alkaline solution of α-naphthol. The burgundy-red to brown-red color, sometimes appearing when ferric chloride, calcium hypochlorite, or chromic acid (3 per cent.) is added to phenacetine-urine, may be due to presence of p-phenetidine.

Phenacetine-urine is frequently intensely yellow and exhibits in consequence of presence of conjugated glycuronic acid laevo-rotation and reduction of Fehling's solution. It differs from urine containing sugar in not fermenting. Detection of p-amino-phenol is more certain, if it is first isolated from urine in the manner

[1] G. Edlefsen: Detection of Phenetidine in Urine. Zentralblatt f. inn. Med. 21 (1900), 2.

described for acetanilide-urine (see page 125) and then examined by the indophenol and azo-dye test.

LACTOPHENINE

Lactophenine is p-lactyl-phenetidine, C_6H_4 (OC_2H_5 (1), $NH.CO.CH(OH).CH_3$ (4))

Preparation.—Heat p-phenetidine lactate, that is, lactic acid and phenetidine, or p-phenetidine with lactic anhydride or the ethyl ester of lactic acid at 150–180°:

$$C_2H_5O.C_6H_4.NH\boxed{H} + \boxed{HO}CO.CH(OH).CH_3 =$$

$$H_2O + C_6H_4 (OC_2H_5 (1), NH.CO.CH(OH).CH_3 (4))$$

Lactophenine forms small, colorless, inodorous crystals having a bitter taste and melting at 117–118°. It is soluble in about 330 parts of cold and in 55 parts of boiling water; and in 8.5 parts of alcohol. These solutions have a neutral reaction.

Toxic Action.—Following use of lactophenine, icterus has been observed with relative frequency. In 9 out of 20 cases icterus appeared after use upon the average of 27 grams distributed over 9 days (S. Laache). The patients were adults, vigorous and usually free from fever. In 77 per cent. of the cases, illness was accompanied by an acute, transient fever. The urine usually had a deep brown color, and in nearly all positive cases gave Gmelin's test for bile-pigment. This test is made by putting in a test-tube 5 cc. of concentrated nitric acid, containing 2 drops of fuming nitric acid in 100 cc., and adding the same quantity of urine, best from a pipette, as an upper layer. If bile-pigment is present, an emerald-green ring appears at the contact-surface of the two liquids. Gradually this ring extends upward and by degrees at the lower part becomes blue, violet-red and yellow. Only the green ring is characteristic of bile-pigment.

According to Rosenbach, pass rather a large quantity of the given urine through a small filter. Allow it to drain well, then open the filter on a glass plate, and spot on the inner side with nitric acid containing some fuming acid. In this manner concentric rings of yellow-red, red, violet, blue and green colors extending outward are obtained.

Skin eruptions may appear, attended with discomfort, temperature in the head, ague, fever, increased pulse and headache. Occasionally blue coloration of the skin probably because of methaemoglobinaemia, with or without collapse, has been observed. The pulse may be quickened and intermittent. Dizziness with disturbances of sight, cloudiness of vision and slight loss of consciousness may produce partial collapse (Lewin).

Detection of Lactophenine

Boiled with hydrochloric acid, lactophenine undergoes hydrolytic cleavage with formation of p-phenetidine which gives the indophenol and azo-dye

test. To differentiate it from phenacetine, the following tests given only by lactophenine may be used:

1. Ferric chloride imparts a reddish brown color to an alcoholic solution of lactophenine.

2. Solution of lactophenine in dilute sulphuric acid even in the cold quickly discharges the color of dilute potassium permanganate solution. If this mixture is gently warmed, odor of acetaldehyde can be detected. This is due to oxidation of lactic acid formed by hydrolytic cleavage:

$$CH_3.CH(OH)COOH + O = CH_3.CHO + H_2O + CO_2.$$

3. Bromine water added to an aqueous solution of lactophenine produces a white precipitate.

Urine.—To detect p-amino-phenol with certainty, this compound should be isolated from lactophenine-urine in the manner described for acetanilide-urine (see page 125) and then detected by the indophenol and azo-dye test.

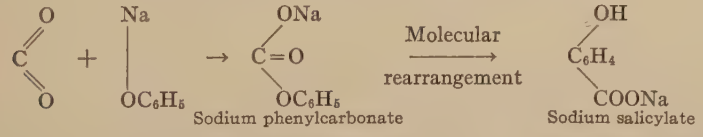
SALICYLIC ACID

Salicylic acid, or o-oxy-benzoic acid, $HO.C_6H_4.COOH$ (1, 2), is fairly wide-spread in the plant kingdom in the free state, also as salicylic aldehyde and methyl ester, and finally as the glucoside salicine. This acid was first isolated from the flowers of Spiraea Ulmaria. As the methyl ester, $HO.C_6H_4.COOCH_3$, salicylic acid occurs in various ethereal oils and as the chief constituent of oil of wintergreen (Gaultheria procumbens).

Preparation.—1. Formerly oil of wintergreen was the source of this acid. When heated with potassium hydroxide solution, it was decomposed into methyl alcohol and potassium salicylate. Acidification of the latter with hydrochloric acid precipitated salicylic acid which finally was crystallized from hot water:

$$HO.C_6H_4.COOCH_3 + KOH = CH_3.OH + HO.C_6H_4.COOK.$$

2. At the present time salicylic acid is prepared from carbolic acid by the method of R. Schmitt. Phenol is dissolved in strong sodium hydroxide solution. Perfectly dry sodium phenolate is obtained by evaporating this solution. This compound is placed in an autoclave, cooled, and saturated under pressure with carbon dioxide. The first product of this reaction is sodium phenyl-carbonate. When this is heated at 120–130°, it undergoes molecular rearrangement and is converted into sodium salicylate frequently used in medicine:



Sodium phenylcarbonate — Sodium salicylate

Properties.—Salicylic acid crystallizes from water in long, white needles having a sweetish, acidulous and rather harsh taste. It is soluble in about 500 parts of cold and in 13 parts of boiling water; and freely soluble in alcohol (1:2), ether

(1:2), chloroform, acetone and carbon disulphide. It melts at 157°. When heated carefully and slowly in the water-bath, salicylic acid sublimes in fine needles. On the other hand, quick heating decomposes it in large part into phenol and carbon dioxide:

$$HO.C_6H_4.COOH = C_6H_5.OH + CO_2.$$

Salicylic acid may also be easily volatilized with steam. Concentrated sulphuric acid dissolves pure salicylic acid in the cold without color and without decomposition. The lead and silver salts of this acid are soluble in water with difficulty. Consequently lead acetate will precipitate lead salicylate, $Pb(OOC.C_6H_4.OH)_2$, from neutral solutions. This salt is white, crystalline and soluble in hot water. It crystallizes unchanged as the hot solution cools. Silver nitrate precipitates white silver salicylate, $HO.C_6H_4.COOAg$.

Physiological Action.—Salicylic acid produces local effects and those resulting from absorption. Locally it acts like a phenol but considerably more feebly than carbolic acid, coagulating albumin and so killing living protoplasm when in very intimate contact. If salicylic acid in pulverulent form is applied to mucous surfaces, it produces white eschars as well as inflammation and necrotic sloughing as a result of this action. Absorption of salicylic acid takes place from all mucous surfaces with such rapidity that it can be detected in the urine even after 15 minutes. The effects of absorption appear in the blood-vessels, then in the central nervous system and kidneys. They arise from injury to protoplasm as in local action. In the kidneys they result from damage to the secreting epithelium (Kobert). Elimination of salicylic acid in the urine begins in the first hour and ends as a rule within 3 days. In addition to elimination in the urine, this acid also leaves the body in perspiration, bile and milk. According to Filippi (La clinica moderna 1900), part of the salicylic acid is said to be eliminated in the synovial fluid of the joints. The brain and spinal cord also withdraw salicylic acid from the blood. Several cases have been described in the literature where severe symptoms of poisoning appeared, following doses of 15, 22 and 50 grams of sodium salicylate. They gave rise to emesis, unconsciousness, delirium, difficulty in hearing, lowering of visual power (mydriasis), albuminuria, fall of temperature, weakness and irregularity of pulse. Recovery, however, took place after a few days. In treatment of articular rheumatism with salicylic acid, the following effects have been repeatedly observed: nausea, emesis, ringing in the ears, faintness of vision, skin eruptions, haemoglobinuria, haematinuria. In urinary sediments have been found: cylinders, epithelial cells of the urinary tubules, and white blood-corpuscles. Addition of salicylic acid as a preservative to foods and condiments is prohibited.

Detection of Salicylic Acid

1. **Ferric Chloride Test.**—Addition of a few drops of ferric chloride solution to an aqueous or alcoholic solution of salicylic acid produces a blue-violet color. If the solution is very dilute, the color is more of a red-violet. By this test salicylic acid is shown to be a derivative of phenol. Excess of the reagent affects the delicacy of the test.

Addition of hydrochloric acid changes the violet color to yellow. Presence of organic acids, such as citric, lactic and tartaric acid, also interferes more or less with this test. This test is not given, if caustic alkalies or alkaline carbonates are present.

2. **Millon's Test.**—If an aqueous salicylic acid solution is warmed with Millon's reagent, a deep red color will appear.

3. **Bromine Water Test.**—In strong excess this reagent produces a yellowish white, crystalline precipitate of tribromo-phenyl hypobromite (see page 44) even with very dilute salicylic acid solutions:

$$HO.C_6H_4.COOH + 8Br = CO_2 + C_6H_2Br_3.OBr + 4HBr.$$

This precipitate should be examined microscopically and its melting-point determined, after it has been filtered and dried upon a porous plate or in a vacuum-desiccator. It melts at 131–132° with evolution of gas.

4. **Methyl Ester Test.**—If salicylic acid is warmed, best in a water-bath, with methyl alcohol and concentrated sulphuric acid, the characteristic odor of methyl salicylate can be recognized:

$$C_6H_4\begin{cases}OH\\COOH\end{cases} + CH_3OH + H_2SO_4 = C_6H_4\begin{cases}OH\\COOCH_3\end{cases} + (H_2O.H_2SO_4).$$

5. **Melting-point Test.**—If the quantity of salicylic acid is not too small, dissolve the ether residue in very little boiling water, shake the hot solution with animal charcoal, filter hot, and allow to crystallize. Determine the melting-point of the crystalline needles, when dry, both direct and after mixing with an equal quantity of pure salicylic acid. In case the quantity permits, make the tests for salicylic acid in 1 to 4. An aqueous distillate should be extracted with a little ether, the ether evaporated, and the residue tested for salicylic acid as described.

Detection of Salicylic Acid in Presence of Simple Phenols

Monacid phenols, such as carbolic acid and the cresols, also give the tests described above in 1 to 3. Separation of carboxylic acids from phenols may be brought about by a cold solution of sodium carbonate which forms salts with acids but not with phenols. To detect salicylic acid in presence of phenols with greater certainty, acidify the material, if neutral or alkaline, with hydrochloric acid, cool and make alkaline with sodium carbonate solution, and completely extract with ether any phenols that may be present. Render the

aqueous solution, containing salicylic acid as the sodium salt, acid with hydrochloric or sulphuric acid. Extract free salicylic acid with ether and test the ether extract.

Directions are given in Chapter V of this book concerning detection of salicylic acid in beer, wine, milk, fruit-juices, meat, sausage-materials, etc., according to the official methods, and also maltol.

Quantitative Estimation of Salicylic Acid as Tribromo-phenyl Hypobromite

(Autenrieth and Beuttel[1])

Place the aqueous solution of salicylic acid in a glass-stoppered flask, add excess of saturated bromine water and shake. The acid is completely precipitated as tribromo-phenyl hypobromite. The solution in the end should be reddish brown. It should stand 12–24 hours and be shaken frequently. Collect the precipitate of tribromo-phenyl hypobromite in a weighed Gooch crucible and dry to constant weight in a vacuum-desiccator (Fig. 13, page 96) over sulphuric acid. The quantity of salicylic acid may be calculated from the weight of precipitate according to the following proportion:

$C_6H_2Br_4O(409.86) : C_7H_6O_3(138.05) =$ Weight of Precipitate $: x$.

Consequently the weight of the precipitate should be multiplied by 0.337.

Detection of Salicylic Acid in Urine[2]

Salicylic acid forms a conjugate with glycocoll, supplied by the organism, and is changed in the human body, in part at least, into salicyluric acid which is eliminated in the urine together with larger quantities of unchanged salicylic acid:

$$HO.C_6H_4.CO.\boxed{OH + H}NH.CH_2.COOH = H_2O + HO.C_6H_4.CO.NH.CH_2.COOH.$$

Such urine gives a violet color with ferric chloride solution. Both salicylic and salicyluric acid give this test. To decompose salicyluric acid into its constituents, heat the acid for half an hour with fuming hydrochloric acid under a reflux:

$$\begin{matrix} C_6H_4.OH \\ | \\ CO.NH.CH_2.COOH \end{matrix} + H_2O + HCl = \begin{matrix} C_6H_4.OH \\ | \\ COOH \end{matrix} + \begin{matrix} CH_2.NH_2.HCl \\ | \\ COOH \end{matrix}$$

To isolate unchanged salicylic acid, acidify 500–1000 cc. of urine with dilute sulphuric acid and repeatedly extract with ether. Remove the ether from the

[1] W. Autenrieth and Fr. Beuttel: Estimation of Phenol, Salicylic Alcohol and Salicylic Acid. Arch. d. Pharm. 248 (1910), 112.

[2] To study the behavior of salicylic acid, take 1.5 grams of sodium salicylate at night in the course of several hours and examine, as described, the urine passed in the next 12–24 hours.

urine in a separating funnel and shake vigorously with excess of sodium carbonate solution. Salicylic acid passes into the aqueous solution, whereas phenols, coloring-matter and other interfering substances remain in the ether. Acidify the sodium carbonate solution with hydrochloric acid and extract salicylic acid with ether. When the ether is distilled or evaporated, it will usually leave this acid in the form of crystals. If necessary, it may be recrystallized from a little boiling water and freed from color by boneblack. A large part of the salicylic acid taken internally can be recovered from human urine. After 3 days elimination is complete.

E. Späth's Procedure.—When a urine gives an uncertain test with ferric chloride, probably very little salicylic acid is present, or substances are causing interference. Evaporate a considerable quantity of the urine upon the water-bath to a small volume, acidify with dilute sulphuric acid, and extract with twice the volume of a mixture of 3 parts of low-boiling petroleum ether and 2 parts of chloroform. According to Späth, this solvent is to be preferred to ether in the examination of foods and condiments, because it contains practically no water and for that reason does not take up substances, such as tannins, that interfere with the ferric chloride test. Pass the solvent through a dry paper, add 1 cc. of water and only a drop of a ferric chloride solution so dilute that the yellow color is just visible, and shake. If salicylic acid is present, the aqueous solution will have a violet color.

SALOL

Of the large number of derivatives of salicylic acid, used medicinally as substitutes for free salicylic acid and its sodium salt, only salol and aspirine have been considered in this book. These two preparations have come into very wide use in medicine. Both possess the valuable therapeutic properties of salicylic acid without its injurious action, that is, without irritating the stomach or exerting other unpleasant secondary effects.

Preparation.—Salol is formed by heating dry salicylic acid for 4 hours in an atmosphere of carbon dioxide at 220–230°:

$$HO.C_6H_4.COOH + HO.C_6H_4.COOH = HO.C_6H_4.COOC_6H_5 + H_2O + CO_2.$$

Consequently salol is the phenyl-ester of salicylic acid.

Salol is a white, crystalline powder having a faint aromatic odor and taste. Its melting-point is approximately 42°. It is almost insoluble in water; soluble in 10 parts of alcohol, 0.3 part of ether, and also in chloroform.

Physiological Properties.—Since salol is an ester, at the temperature of the body, in contact with the secretion of the pancreas, with the living mucous membrane of the intestines and other living tissues, it undergoes decomposition into phenol and salicylic acid:

$$HO.C_6H_4.COOC_6H_5(+ \text{Enzyme}) + H_2O = HO.C_6H_4.COOH + C_6H_5.OH.$$

Therefore symptoms of intoxication, due to both these cleavage-products, may appear. Both occur in the urine. For this reason salol-urine possesses the properties of carbolic-urine. It gradually acquires an olive-green color, becoming finally brown and brown-black. A man 40 years old who had taken

22.5 grams of salol in 4 days had a strong albuminuria and severe pain in the region of the kidneys. A girl 22 years old after taking 8 grams of salol became drowsy, fell into a coma, and the perspiration had an aromatic odor. Death ensued on the third day. The autopsy revealed acute and profound degeneration of the epthelium of the kidneys. Where individuals have used salol externally in the form of ointments, typical intoxications, especially diseases of the skin, have appeared. In two instances where powdered salol was blown into the nose, eczema was developed in the nostrils and upon the upper lip. In a third case the eczema extended over a large part of the face. A vaseline ointment containing 10 per cent. of salol was responsible for this result.

Detection of Salol

An alcoholic solution of salol, but not an aqueous, filtered extract, takes on a dirty violet color with very dilute ferric chloride solution. If 0.2–0.3 gram of salol is brought into solution by warming with as little sodium hydroxide solution as possible, sodium phenolate and sodium salicylate are formed. Crystals of salicylic acid separate and the odor of phenol can be recognized, when this solution is cooled and acidified with hydrochloric acid:

(α) $HO.C_6H_4.COOC_6H_5 + 2NaOH = HO.C_6H_4.COONa + C_6H_5.ONa + H_2O$,
(β) $HO.C_6H_4.COONa + C_6H_5.ONa + 2HCl = HO.C_6H_4.COOH + C_6H_5.OH + 2NaCl$.

To separate the two products of this reaction, extract first with ether and then shake the ether solution with sodium carbonate solution. Phenol remains dissolved in the ether, whereas salicylic acid goes into the sodium carbonate solution as the sodium salt. If the latter is acidified with hydrochloric acid, ether will now extract only salicylic acid. The residues obtained by distilling or evaporating these two ether solutions may be tested in the manner already described for phenol and salicylic acid.

Similarly salol-urine may be acidified with hydrochloric acid and extracted with ether. The residue from this ether solution can be tested for the two cleavage-products, that is, phenol and salicylic acid, formed in the animal organism, or for salicyluric acid resulting from salicylic acid.

ASPIRINE

Aspirine, or aceto-salicylic acid, $CH_3.CO.OC_6H_4.COOH$ (1, 2), is formed by the action of acetic anhydride or acetyl chloride upon salicylic acid:

$(CH_3.CO)_2O + HO.C_6H_4.COOH = CH_3.COOH + CH_3.CO.O.C_6H_4.COOH$.
$CH_3.CO.Cl + HO.C_6H_4.COOH = HCl + CH_3.CO.O.C_6H_4.COOH$.

Aspirine is a white, inodorous, crystalline powder having a sweet, acidulous taste and melting at about 137°. It is soluble in 100 parts of water, 4.5 parts of alcohol, 10 parts of ether, and 26 parts of chloroform. An aqueous aspirine solution has an acid reaction. Ether extracts aspirine from an acid aqueous solution. Heated with potassium or sodium hydroxide solution, or even with water, it is hydrolyzed into salicylic acid and acetic acid:

$CH_3.CO.O.C_6H_4.COOH + H_2O = CH_3.COOH + HO.C_6H_4.COOH$.

Physiological Properties.—Aspirine irritates the mucous membrane of the stomach but slightly and like salol reaches the intestines without decomposition. There it is only slowly resolved into its two components. The remainder is decomposed after absorption. Its elimination is slower than that of sodium salicylate. The urine contains salicylic and salicyluric acid. Poisonings from aspirine with fatal outcome have probably not occurred. Repeatedly, mild toxic symptoms have been observed after doses of 0.5–1 gram of aspirine, that is, oedematous swellings, especially of the eyelids, and of the forehead and scalp. But they have usually disappeared quickly. Severer symptoms of poisoning have been observed after the administration of aspirine to children: drowsiness, coma, emesis, acetone in the breath and urine.

Detection of Aspirine

Heat 0.2–0.5 gram of aspirine for a few minutes with a little sodium hydroxide solution, cool, best by setting the test-tube in ice-water, and acidify with dilute sulphuric acid. After some time filter off the salicylic acid that crystallizes out and apply the tests already described for its recognition. The second component of the hydrolysis of aspirine, that is, acetic acid, can usually be recognized by its odor in the filtrate from salicylic acid. To detect it with greater certainty, warm the filtrate with some alcohol and concentrated sulphuric acid. The odor of acetic ether will be developed. To detect salicylic and salicyluric acid in urine, test a portion with ferric chloride. If the result is not convincing, proceed according to the method (see page 134) employed in detecting salicylic acid in urine.

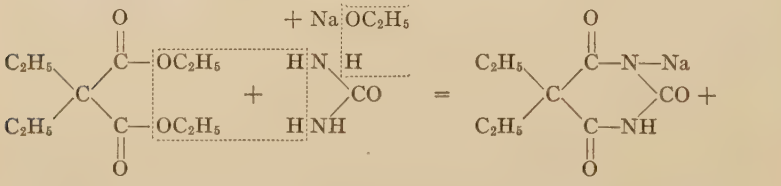
VERONAL

Veronal, or barbital (name adopted by the Federal Trade Commission for the substance formerly sold under the protected name "Veronal"), is C-diethyl-barbituric acid, C-diethyl-malonyl-urea, $C_8H_{12}O_3N_2$. It crystallizes from hot water in large, colorless, spear-shaped crystals melting at 191° (corrected) and is soluble in 146–147 parts of water at 20° and in 15 parts at 100°. Veronal is also freely soluble in hot alcohol and acetone. It dissolves with difficulty in cold ether, benzene and chloroform. Heated above its melting-point, veronal sublimes in long, shining needles almost without decomposition. An aqueous veronal solution has a bitter taste and shows a very faint acid reaction with sensitive blue litmus paper. Veronal dissolves easily in caustic alkalies, ammonia, and in calcium or barium hydroxide solution, but less easily in cold alkaline carbonate solutions. From such solutions, provided they are not too dilute, acids precipitate veronal unchanged and usually crystalline. Of the veronal salts, the sodium salt, $C_8H_{11}O_3N_2Na$, crystallizes best. It may be prepared by dissolving veronal in the calculated quantity

of caustic soda solution free from carbonate, and then evaporating this solution with exclusion of carbon dioxide, or by adding alcohol until turbidity appears. In both cases the sodium salt of veronal, also called "Medinal," separates as splendid shining crystals.

Preparation by Fischer and Dilthey[1]

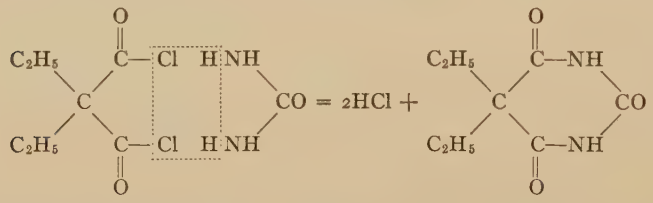
(a) From diethyl-malonic ester by condensation with urea in presence of sodium ethylate:



Diethyl-malonic ester | Urea | Sodium salt of veronal

Dissolve metallic sodium (32 parts) in absolute alcohol (600 parts) and when cold add diethyl-malonic ester (100 parts). Dissolve in this mixture with heat finely powdered urea (40 parts). Heat 4–5 hours in an autoclave at 105–108°. The sodium salt of veronal is precipitated even from the hot solution as a colorless, crystalline mass. Cool, filter with suction and wash with alcohol. Dissolve the crystals in water and acidify with concentrated hydrochloric acid. Veronal thus precipitated is pure when recrystallized from water.

(b) From diethyl-malonyl chloride by condensation with urea:



Heat diethyl-malonyl chloride (3 parts) upon the water-bath for 20 hours with finely powdered, dry urea (2 parts). Considerable hydrogen chloride is given off toward the end and a solid mass finally remains that yields pure veronal upon crystallization from hot water. The yield is 70 per cent. of the theoretical amount.

Toxic Action.—Veronal does not cause decomposition of the blood and in the usual medicinal doses (0.5–1 gram) does not appear to act strongly upon the heart. Cumulative action has been noted only in rare instances. In rather large doses, however, veronal may cause serious intoxication with fatal termina-

[1] E. Fischer and A. Dilthey: C-Dialkyl-barbituric Acids and the Ureids of Dialkyl-acetic Acids. Annalen d. Chemie 335 (1904), 334.

tion. The opinion in general appears to be that 10 grams of veronal is a fatal dose for adults. The symptoms of acute veronal poisoning resemble those of morphine poisoning: abnormally deep sleep, prolonged unconsciousness, cyanotic face, feeble respiration, retching, tetanus-like convulsions, extremities cold and sluggish, contracted and unreactive pupils make up the usual picture of acute veronal poisoning. The following case shows the course of a veronal- (medinal-) poisoning.

Autenrieth[1] recovered from the urine of a girl, who had taken 20 tablets each 0.5 gram of medinal at a dose, 3.2 grams of nearly pure veronal. After 36 hours of sleep, 400 cc. of urine were drawn from the bladder by catheter. The physician, who had charge of the girl, gave the author the following record of the case:

"Twenty medinal tablets were dissolved in water and taken at a dose. About 10 hours later there was deep coma, complete anaesthesia, pupils highly contracted, showing scarcely any reaction; otherwise no reflexes; general paresis; pulse 120, low but regular; respiration 32, regular. Later on: pulse up to 150, regular; respiration up to 75, very feeble, somewhat irregular (30 to 34 hours after the poisoning). Following injections of camphor, pulse and respiration improved! After the effect of the camphor had worn off, improvement disappeared. After 70 hours there was still only a very slight quickening of pulse and respiration. After 44 hours there was a very faint corneal reflex. After 54 hours there were slight muscular movements. Unconsciousness lasted for 60 hours! After 66 hours consciousness was tolerably clear. Temperature 38.6 to 39.1°; quick drop to 36.8°, following a spontaneous stool after 54 hours. Retention of urine during 54 hours. Elevated, urticaria-like, red spots appeared upon the inner surface of the legs upon the impressions of the condyles. These did not completely disappear for a week. Upon return of consciousness after 66 hours: pressure in the head, slight stupor which, however, quickly passed away. During 4–5 days there was muscular uneasiness and pains in the limbs. After 5 days the urine was again free from albumin."

This case shows that veronal is a sure and also tolerably safe hypnotic. The 20 medinal tablets, taken in aqueous solution at one dose by this 18 year old girl, corresponded to 8 grams of veronal. On the other hand, 2 cases of suicide from veronal are known. In one case 11 grams and in the other 15 grams of veronal were taken at a dose. In the latter case after 15 minutes there was unconsciousness and contraction of the pupils. Atropine again dilated the pupils but otherwise was of no avail. Death ensued after 20 hours. Only in the severest cases of veronal poisoning do the pupils contract and become fixed as in acute morphine poisoning.

Detection of Veronal

1. Veronal may be sublimed almost without decomposition when heated in a test-tube and well-formed crystals are obtained. The aqueous solution of these veronal crystals reacts faintly acid to litmus paper.

[1] W. Autenrieth: A Case of Medinal Poisoning and Detection of Veronal in the Urine. Ber. d. Deutsch. pharmaz. Ges. 31 (1921) Heft 3.

2. Veronal is soluble in solutions of potassium or sodium hydroxide, ammonia and sodium carbonate. If these solutions are as nearly saturated as possible, dilute hydrochloric acid will precipitate veronal unchanged.

3. The melting-point of pure veronal is 187–188°. If the substance suspected of being veronal is mixed with the same quantity of pure veronal, the melting-point should not change.

4. With Millon's reagent, or a solution of 1 gram of yellow mercuric oxide in 2 cc. of nitric acid, as well as with Denigès reagent, an aqueous solution of veronal gives a white, gelatinous precipitate soluble in a large excess of Millon's reagent.

Detection of Veronal in Cadaveric Material

In examining cadaveric material (liver, spleen and kidneys) from a man, who had taken veronal, thinking it was a remedy for tapeworm (kamala), G. and H. Frerichs[1] isolated small quantities of the drug. Following the Stas-Otto process, they extracted the aqueous tartaric acid solution with ether and evaporated the ether extract. They recrystallized the residue from a little hot water, using animal charcoal to remove color, and identified the crystals obtained as veronal by the tests given above. In Chapter V of this book (page 567) the subject of veronal or medinal poisoning has received special treatment under the heading "Detection and Estimation of Veronal in Urine."

Apparently veronal, as Panzer[2] states, is not retained in the cadaver as such longer than 4 weeks. It may also happen in veronal poisoning that not a trace of the poison can be found in the cadaver. Such may be the case, for example, when death has not ensued for quite a long time after the last dose of veronal was taken and consequently all the poison has been eliminated through the kidneys.

In examining by the Stas-Otto process freshly removed organs of a woman, who had died under the suspicion of having been poisoned, Heiduschka[3] found only very small quantities of veronal:

Quantity	Material	Veronal
100 cc.	Urine	0.0246 gram
200 grams	Parts of liver, lungs, spleen and heart	0.014 gram
200 grams	Parts of oesophagus, stomach and contents, large and small intestine, and bile	0.0079 gram

[1] G. and H. Frerichs: Detection of Veronal Poisoning. Archiv d. Pharmaz. 244 (1906), 86.

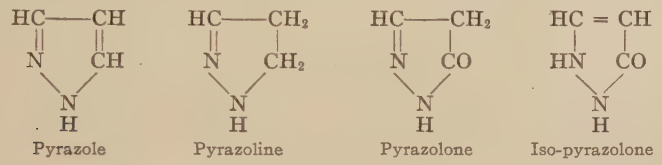
[2] Th. Panzer: Detection of Veronal. Vierteljahrsschr. f. gerichtl. Medizin (3) 36 (1908), 311.

[3] A. Heiduschka: Forensic Detection of Veronal. Archiv d. Pharmaz. 249 (1911), 322.

It was to be expected that larger quantities of the hypnotic would be found in the urine but this did not turn out to be true in the case examined by Heiduschka. Apparently it was one of those cases, the possibility of which has been pointed out by Panzer, where death from veronal poisoning has not occurred until most of the drug has been elminated from the body.

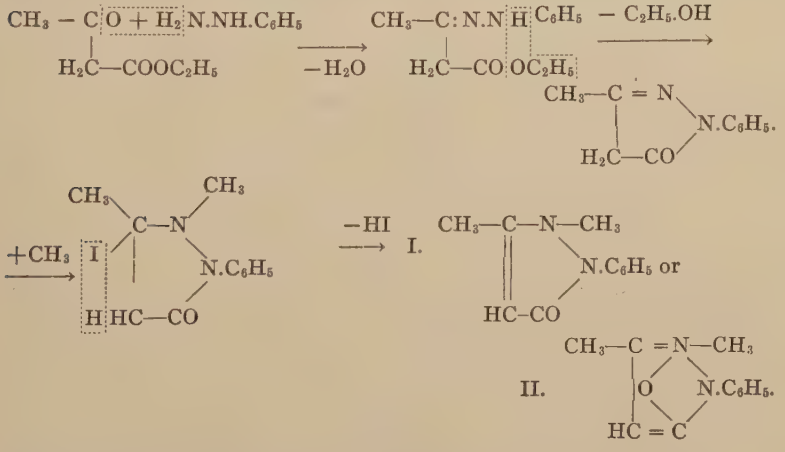
ANTIPYRINE

Antipyrine, according to its discoverer Knorr (1884), is to be regarded as a derivative of pyrazole, that is, of iso-pyrazolone. All derivatives of pyrazole contain a five-atom, heterocyclic ring consisting of 3 carbon and 2 nitrogen atoms:

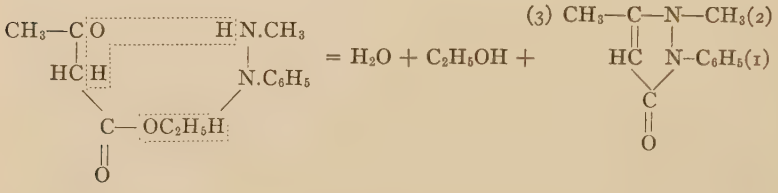


Iso-pyrazolone is tautomeric with pyrazolone and is known only in the form of alkyl and phenyl-derivatives.

Preparation.—I. The oily phenyl-hydrazine-aceto-acetic ester, formed by mixing phenyl-hydrazine and aceto-acetic ester, is separated from the water of the reaction and warmed in the water-bath until a test-portion solidifies upon cooling. The phenyl-methyl-pyrazolone thus obtained is washed with ether and heated at 100–110° with methyl iodide in methyl alcohol solution. The methyl iodide addition-product formed is treated with sodium hydroxide solution. Antipyrine is formed with cleavage of hydriodic acid and rearrangement of the pyrazolone into the iso-pyrazolone ring:



II. Antipyrine is formed direct by heating aceto-acetic ester with symmetrical methylphenyl-hydrazine:



Crude antipyrine is purified by crystallization from ether, ligroin or toluene.

Properties.—Antipyrine forms monoclinic, tabular crystals having a bitter taste and melting at 113°. One part of antipyrine is soluble in less than 1 part of cold water, in about 1 part of alcohol, 1 part of chloroform, and 50 parts of ether. An aqueous antipyrine solution has a neutral reaction to litmus, although this compound forms salts with acids that usually crystallize well.

Physiological Properties and Secondary Effects.—In doses of 1–2 grams antipyrine acts as an antipyretic, causing a drop in temperature within 4 hours frequently with copious sweating. Antipyrine is also valuable as a sedative and anodyne. According to Meyer and Gottlieb ("Pharmakologie" 1920), secondary effects rarely appear when the dose is not too large, that is, less than 2.0 grams. A very remarkable fact is the idiosyncrasy exhibited by many persons for antipyrine. The most frequent secondary effect is an antipyrine exanthema which is troublesome but not dangerous. More severe skin affections occur only in case of idiosyncrasy, such as inflammatory swelling of the skin of the face, also the skin of the genitals; and symptoms of severe irritation of mucous surfaces, that is, conjunctivitis, catarrh of the nose, throat and larynx. More frequently there is also nausea, retching and vomiting, rarely bloody vomitus, pains in the abdomen, and diarrhoea. The most frequent secondary effect connected with the urino-genital organs is a decrease in the secretion of urine; the appearance of albumin and sugar has also been observed in individual cases. Derangements of the respiratory organs appear in various forms: oppression and pains in the chest, painful respiration with a feeling of suffocation may occur in connection with symptoms of irritation in the nose and larynx, the appearance of which is probably attributable to the same anatomical changes, that is, swelling of the mucous surfaces of the respiratory passages (Lewin).

Detection of Antipyrine

1. Ferric Chloride Test.—Add 1–2 drops of dilute ferric chloride solution to an aqueous antipyrine solution. It will produce a deep red color that can be seen even in a dilution of 1:100,000. Dilute sulphuric acid changes the red to a pale yellow color.

2. **Tannic Acid Test.**—Tannic acid solution produces a white precipitate, when added to an aqueous antipyrine solution. Obviously this test is not characteristic of antipyrine.

3. **Fuming Nitric Acid Test.**—Add 1–2 drops of fuming nitric acid to an antipyrine solution. The solution will turn green. Then heat to boiling and add another drop of fuming nitric acid. The green color will change to red. This test is distinctly given by 1 cc. of an aqueous antipyrine solution (1:200).

4. **Nitroso-antipyrine Test.**—Add a few drops of potassium or sodium nitrite solution to an aqueous antipyrine solution and then dilute sulphuric acid. A green or blue color will appear. A few drops of acetic acid may be substituted for sulphuric acid but the solution must be heated. With concentrated antipyrine solutions green crystals of nitroso-antipyrine, $C_{11}H_{11}(NO)ON_2$, will separate after some time.

Detection of Antipyrine by the Stas-Otto Process

Ether extracts only small quantities of antipyrine from a solution containing much tartaric acid. Ether, or better chloroform, extracts by far the greater part of the antipyrine when the solution has been made alkaline with sodium hydroxide solution or ammonia. If the material contains notable quantities of antipyrine, usually it will appear in all ether and chloroform extracts in the Stas-Otto process. Antipyrine differs from most of the alkaloids by its solubility in water. To detect antipyrine, dissolve the residue from the ether or chloroform extract in a little water and examine this solution by the tests described above. Of the general alkaloidal reagents, picric acid and potassium bismuthous iodide are especially adapted for the precipitation of antipyrine. Antipyrine may be weighed in the form of the yellow crystals of antipyrine picrate, $C_{11}H_{12}ON_2.C_6H_2(NO_2)_3(OH)$.

Detection of Antipyrine in Urine

Antipyrine is easily absorbed and rapidly distributes itself in the human organism, so that it can be detected in all organs at once. Its elimination through the kidneys is proportionately rapid and it occurs in the urine partly unchanged and partly conjugated with sulphuric acid. Antipyrine-urine has a yellow to blood-red color and according to Penzoldt sometimes exhibits dichroism, red in transmitted and green in reflected light. In such a urine antipyrine usually can be detected direct with ferric chloride. To detect antipyrine with greater certainty, especially when the urine has a very dark color or contains

aceto-acetic acid, as is frequently the case in diabetes mellitus, render a considerable quantity of the urine strongly alkaline with ammonia or sodium hydroxide solution. Then extract with chloroform, pour this extract through a dry filter, and evaporate or distil off the solvent. Dissolve the residue in a little water and test the filtered solution for antipyrine with ferric chloride and with fuming nitric acid. Antipyrine is so easily absorbed that even an hour after it has been taken it will impart a reddish color to the urine and may be detected by the ferric chloride test. After about 25 hours the red color of the urine disappears but elimination after a dose of 2–3 grams of antipyrine continues for 36 hours. After a dose of 5 grams of antipyrine, no test is given at the end of about 50 hours. Jonescu[1] recommends a zone-test for urine by adding very dilute ferric chloride solution as an upper layer. If the urine contains antipyrine, a fine red ring will appear. In doubtful cases repeat the test with normal urine free from antipyrine. Moderate doses of antipyrine, 1–2 grams daily, apparently have no influence upon sulphur-metabolism, whereas larger doses, 5 grams and more, seem to diminish sulphate-sulphuric acid somewhat and to increase somewhat more ethereal sulphuric acid. Small doses of antipyrine are eliminated in the urine unchanged and only after larger doses does partial conjugation with sulphuric acid occur. In this manner the organism protects itself against larger doses by conjugation with a certain amount of the antipyrine, whereas the remainder passes off unchanged in the urine. To detect antipyrine itself in urine, Jonescu recommends precipitation by means of potassium bismuthous iodide solution suggested by Thoms. For this purpose acidify the urine with sulphuric acid, precipitate with the bismuth reagent, filter the precipitate by suction, and wash well with faintly acidulated water. Then triturate the precipitate with crystallized sodium carbonate and 10 per cent. sodium hydroxide solution, and extract thoroughly with chloroform in a glass-stoppered separating funnel. Distil or evaporate the solvent. Antipyrine usually gives a crystalline residue. The purine-bodies of normal urine are also precipitated by the bismuth reagent but they do not give a residue with chloroform. Gadamer recommends silver carbonate for the decomposition of the bismuth precipitate.

Quantitative Estimation of Antipyrine as Picrate

(a) **Gravimetric Estimation.**—According to Riedel,[2] antipyrine (Mol. Wt. 188) may be weighed as the picrate, $C_{11}H_{12}ON_2.C_6H_2(NO_2)_3OH$ (Mol. Wt. 417). For this purpose, dissolve 0.5 gram of antipyrine, or an antipyrine mixture corresponding approximately to this quantity, in 50 cc. of water. Add 5–6 cc. of n-hydrochloric acid, heat to boiling, and add 10 cc. of a cold saturated alcoholic solution of picric acid. Allow to stand in the cold for several hours (preferably until the following day) and collect the precipitated antipyrine picrate crystals in a weighed Gooch crucible, adding by rinsing those crystals that adhere to the sides of the beaker used for precipitation. Remove completely all liquid by suction and dry crucible and precipitate at about 95° to constant weight in a steam drying-closet. Washing the crystals with water, alcohol, etc.,

[1] D. Jonescu: Elimination of Antipyrine from the Human Organism. Ber. d. Deutsch. pharm. Ges. 16 (1906), 133.

[2] J. D. Riedel: Riedel's Berichte 1907, page 67.

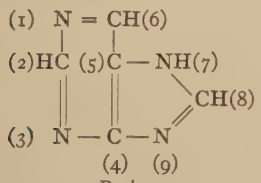
is not permissible on account of their solubility. Since the quotient Antipyrine: Picrate = 188:417 = 0.451, the quantity of antipyrine is obtained by multiplying the weight of precipitate by 0.451. As a check, the melting-point of the crystals should be determined. Pure antipyrine picrate melts at 187°.

(b) **Volumetric Estimation.**—Lémaire[1] precipitates antipyrine in aqueous solution with excess of 0.05 n-picric acid solution and measures in an aliquot part of the filtrate from the antipyrine picrate precipitate excess of picric acid with 0.1 n-sodium hydroxide solution, using phenol-phthalein as indicator. A liter of 0.05 n-picric acid solution contains 0.05 $C_6H_2(NO_2)_3(OH) = 229:20 =$ 11.45 grams of pure picric acid. This quantity of picric acid will precipitate 0.05 $C_{11}H_{12}ON_2$ grams = 188:20 = 9.4 grams of antipyrine.

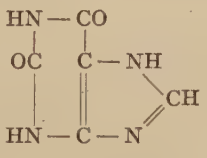
Procedure.—Put 10 cc. of an approximately 5 per cent. aqueous antipyrine solution in a 100 cc. graduated flask, fill to the mark with 0.05 n-picric acid solution, that is, with 90 cc., shake well, allow to stand in the cold for 0.5–1 hour, and filter 50 cc. through a dry paper into a dry graduated flask. Now add 1 cc. of phenol-phthalein solution and titrate with 0.1 n-sodium hydroxide solution to a permanent red color. If the number of cc. of 0.1 n-alkali used is quadrupled and 90 is subtracted from the number obtained, it will give the number of cc. of 0.05 n-picric acid combined with the antipyrine in the 10 cc. taken.

CAFFEINE

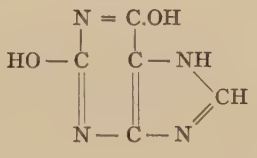
Caffeine together with theophylline and theobromine constitute a group of closely related substances derived from xanthine by substituting methyl for two or three atoms of hydrogen. Caffeine is 1, 3, 7-trimethyl-xanthine, theophylline, 1, 3-dimethyl-xanthine, and theobromine, 3, 7-dimethyl-xanthine. All these substances are derivatives of purine. Their close relation to purine is shown by their structural formulae:



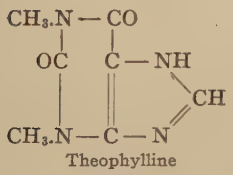
Purine



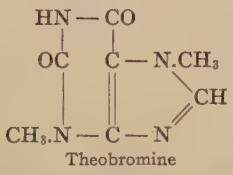
Xanthine (Keto-form)



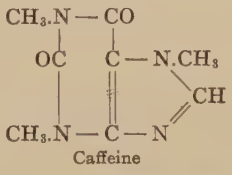
Xanthine (Enol-form)



Theophylline



Theobromine



Caffeine

Properties.—Caffeine, also called theine, crystallizes in white, shining needles. It is soluble in 80 parts of water, giving a colorless solution with a neutral reac-

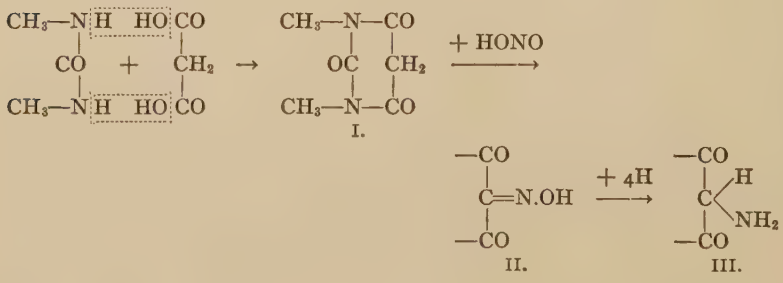
[1] P. Lémaire: Répertoire de Pharmacie 1904, No. 11.

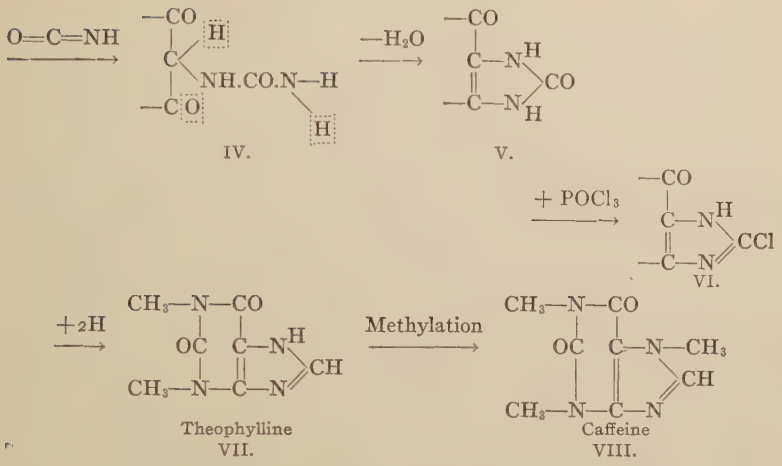
tion and a bitter taste. It is quite easily soluble in hot water (1:2). It requires for solution about 50 parts of alcohol, 9 parts of chloroform, but is only slightly soluble in ether, absolute alcohol, benzene and petroleum ether. In crystallizing from hot water, caffeine combines with 1 molecule of water, a part of which it loses upon exposure to air and all when dried at 100°. It melts at 230°, but somewhat above 100° begins to volatilize in small quantity and at 180° to sublime in colorless needles without leaving a residue. Concentrated sulphuric and nitric acid dissolve caffeine without color. Caffeine is a very weak base and its salts are decomposed by water. Therefore, caffeine can be extracted at least partially by ether, or better by chloroform, from an aqueous tartaric acid solution.

Synthetic Caffeine.—The synthesis of caffeine was first accomplished by heating the silver salt of theobromine for a long time at 100° with methyl iodide. This reaction proceeds more smoothly, if theobromine (1 Mol.) and potassium hydroxide (1 Mol.) in alcoholic solution are heated with methyl iodide (1 Mol.) in a closed vessel for 6 hours at 100° (E. Schmidt); or if the solution of theobromine in excess of sodium hydroxide solution is shaken with dimethyl sulphate, $(CH_3O)_2SO_2$. In the same manner theophylline and paraxanthine, or 1,7-dimethyl-xanthine, may be converted into caffeine by methylation. Quite a remarkable synthesis of caffeine was effected by Emil Fischer. Starting with α, β-dimethyl-urea, obtained from methyl cyanate and methyl amine:

$$O=C=N.CH_3 + NH_2.CH_3 \rightarrow O = C(NH.CH_3)_2$$

he condensed it with malonic acid to dimethyl-malonyl urea, or dimethyl-barbituric acid (I). This compound was converted by nitrous acid into dimethyl nitroso-barbituric acid (II). The latter upon treatment with nascent hydrogen, that is, by the action of hydriodic acid, gave dimethyl-uramil (III). Since this compound is a primary amine, it adds cyanic acid in the form of its potassium salt, yielding dimethyl-pseudo-uric acid (IV). Elimination of water and ring-closur takes place, when the free acid is heated with anhydrous oxalic acid, and dimethyl-uric acid (V) is formed. Treatment of the latter with phosphorus oxychloride ($POCl_3$) converts it into chlorotheophylline (VI). Reduction by means of hydriodic acid substitutes hydrogen for chlorine and gives theophylline (VII). This synthetic 1, 3-dimethyl-xanthine has been shown to be identical in every respect with natural theophylline occurring in tea. By methylation theophylline is converted into caffeine (VIII):





Physiological Action.—In small doses caffeine acts as a stimulant upon the central nervous system so that the efficiency of the brain and spinal cord is increased. The agreeable effects produced by drugs containing caffeine are due to this property. Their irritability is increased by larger doses of caffeine to such an extent that it amounts to tetanus. Aside from that, caffeine gives rise to a muscular change, so that the striped muscles become as rigid as in death. Caffeine acts as a powerful diuretic. Inasmuch as diuresis is also excited even in the excised kidneys, it cannot be dependent, at least not exclusively, upon changes in blood-pressure.

The symptoms of intoxication, appearing in acute poisoning by caffeine, are like those resulting from the state of excitation occasioned by drunkenness: dizziness, ringing in the ears, headache, trembling, uneasiness, sleeplessness, confusion of ideas, delirium, palpitation of the heart, albuminuria, stiffness of the muscles, and even real convulsions. Death ensues as a result of paralysis of the heart. Kobert cites the case of a man who had taken about 4 grams of caffeine. After 15 minutes there occurred: burning in the chest, trembling, dizziness, emesis and diarrhoea, marked diuresis, abdominal pains, highly accelerated pulse and collapse. After 9 hours collapse subsided and 3 days later the patient was again quite normal. But even after a dose of 0.5–0.6 gram of caffeine, a condition of excitation like that of drunkenness, sleeplessness, dizziness, muscular tremors, inclination to emesis and diarrhoea, as well as severe micturition may also appear. After larger doses of about 1 gram, in addition to these symptoms, palpitation of the heart appears, considerable increase in the frequency of the pulse and irregularity in the heart's action. Without severer secondary effects, caffeine poisoning is accustomed to disappear. (Hans Meyer-Gottlieb.)

Elimination of Caffeine.—Only a very small part of the caffeine, and then only when larger quantities of it have been taken, passes through the organism unchanged and appears as such in the urine. Another part of the caffeine, about 10 per cent. of that taken, undergoes demethylation in metabolism, since

mono- and dimethyl-xanthine appear in the urine. The remainder of the caffeine must undergo conversion into the normal end-products of human metabolism.

Detection of Caffeine

Ether will extract more caffeine from an aqueous alkaline solution than from an aqueous tartaric acid solution. Since caffeine dissolves with some difficulty in ether, but more easily in chloroform, the latter solvent is usually employed after the solution has been made alkaline with ammonia. After distillation of solvent, caffeine appears in concentric clusters of long, shining needles. In an analysis by the Stas-Otto method, caffeine will appear in all three extracts. Chloroform, benzene and also amyl alcohol extract caffeine completely from acid solution, whereas petroleum ether will extract it neither from acid nor alkaline solution. Caffeine in dilute sulphuric acid solution (1:1000) is precipitated only by such general alkaloidal reagents as phospho-molybdic acid, phospho-tungstic acid, and potassium bismuthous iodide.

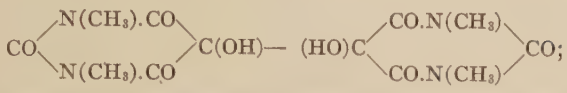
1. **Amalic Acid Test.**—Gradually evaporate caffeine to dryness in a small dish upon the water-bath with a few cc. of strong chlorine water,[1] taking about 10 times the quantity of the latter. A red to red-brown residue will remain. If a very little ammonium hydroxide solution is at once added, a fine purple-violet color will appear. This test may be made by covering the dish containing the residue with a glass plate moistened with a drop of strong ammonia. Or two matched watch-glasses may be used, the material containing caffeine being evaporated to dryness with chlorine water upon one glass which is then placed for a short time upon the other glass containing a drop of strong ammonia.

This test is not confined to caffeine, for it is also given by theophylline and theobromine. They, however, require rapid evaporation with about a hundred times the quantity of chlorine water. The residue left upon evaporation is also red but the shades of color that appear with ammonia are somewhat different. But caffeine may be separated from theophylline and theobromine by the fact that benzene will extract it from alkaline solution and leave the other two bases behind. If the solution is then acidified, chloroform will extract theophylline and theobromine. According to Gadamer, theophylline further differs from caffeine and theobromine in giving a red color with the diazonium reagent.[2]

[1] A convenient method of preparing saturated, aqueous chlorine solution is to heat potassium chlorate with hydrochloric acid and pass the chlorine into a small quantity of water.

[2] See "Preparation of Reagents" page 641.

Amalic acid is tetramethyl-alloxantine,



and so is formed from 2 molecules of caffeine or theophylline with cleavage of the remainder of the molecule, that is, —$N(CH_3).CH:N$— or —$NH.CH:N$—. Amalic acid forms colorless crystals that easily redden in the air, are difficultly soluble in water and insoluble in absolute alcohol. In contact with ammonia, amalic acid takes on a purple-red color and a blue color when moistened with potassium or sodium hydroxide solution.

2. **Tannic Acid Test.**—This reagent, added to an aqueous caffeine solution, causes a heavy white precipitate soluble in an excess of the acid. This test is not characteristic of caffeine.

B. Examination of Ether Extract of Aqueous Alkaline Solution

This ether extract may contain alkaloids and other basic substances. The exceptions are the phenolic bases, morphine, apomorphine and narceine.

The aqueous tartaric acid solution withdrawn in a separating funnel from the last ether extract should be made strongly alkaline with sodium hydroxide solution.[1] The alkali will liberate alkaloids from their salts and combine with the phenolic bases. Then thoroughly extract this solution at once with about the same volume of ether. Separate the ether from the aqueous solution and again extract with a fresh quantity of ether. Withdraw the latter also. In certain cases 3–4 such extractions may be required. This is necessary when a substance like strychnine, that is difficultly soluble in ether, is under examination. Mix the combined ether extracts in a dry flask, stopper loosely and set aside for 2–3 hours. Carefully decant the ether through a dry paper and evaporate (**Extinguish all flames!**) in a small glass dish upon a water-bath that has previously been warmed.

[1] When the examination is concerned exclusively with one of the "ester-alkaloids," such as atropine, hyoscyamine, cocaine, scopolamine, or with physostigmine, or with the lactone, pilocarpine, but not with morphine or apomorphine, it is better to render the solution alkaline with sodium carbonate, or better bicarbonate, rather than with sodium hydroxide solution. They will set the alkaloids free from their salts without causing saponification or cleavage after that has taken place:

$$(C_{17}H_{23}O_3N)_2.H_2SO_4 + Na_2CO_3 = 2C_{17}H_{23}O_3N + H_2O + CO_2 + Na_2SO_4.$$

Atropine sulphate — Atropine

Larger quantities of ether should first be distilled to a volume of 10–15 cc. and this more concentrated ether solution then evaporated in a glass dish as described above. Special precaution should be taken not to allow even a drop of the aqueous solution, that usually separates in small quantity when the ether extract is allowed to stand in a flask, to mix with the filtered ether solution. Moreover the residue from the ether solution should not be allowed to stand for a needlessly long time upon the warm water-bath, otherwise alkaloids that may be present have a tendency to become viscous and coniine and nicotine may volatilize.

The residue from the ether extract of the aqueous alkaline solution may contain nearly all the alkaloids with the exception of the phenolic bases, morphine, apomorphine and narceine, as well as other organic substances that are basic in character. It should be examined for

Coniine	**Atropine**	**Hydrastine**
Nicotine	**Scopolamine**	**Pilocarpine**
Aniline	**Cocaine**	**Quinine**
Veratrine	**Physostigmine**	**Caffeine**
Strychnine	**Codeine**	**Antipyrine**
Brucine	**Narcotine**	**Pyramidone**

First, examine the appearance of the residue both macroscopically and microscopically. Taste it cautiously. Certain alkaloids may be recognized beforehand by this test. Tests for any such substances in the residue should be made first.

The various alkaloids appear in the residue as follows:

Strychnine.—Very fine needles having an exceedingly bitter taste.

Brucine.—White, amorphous powder having a very bitter taste.

Veratrine.—Amorphous powder having a sharp, burning taste.

Atropine, Quinine and Codeine.—Varnishes that are usually resinous and sticky but rarely crystalline.

Caffeine.—Long, frequently concentrically arranged needles having a faintly bitter taste.

Pyramidone.—Fine, crystalline needles easily soluble in water and having a faintly bitter taste.

Scrape the residue together with a platinum or nickel spatula, or with the clean blade of a pocket-knife, to get some idea as to the quantity of alkaloid present.[1]

[1] The author evaporates ether and chloroform extracts upon accurately weighed watch-glasses, removing the last 5–6 cc. of solvent in the vacuum apparatus shown upon pages 96 and 111.

Preliminary Tests for Nitrogenous Bases

Dissolve a test-portion of this residue in 2–3 cc. of water, adding a drop of dilute hydrochloric acid. Filter this solution, if necessary, distribute it in several small test-tubes, and add to each portion one of the general alkaloidal reagents, such as mercuric chloride, potassium mercuric iodide, potassium bismuthous iodide, picric acid, phospho-molybdic acid and phosphotungstic acid. If an alkaloid is present, abundant precipitates will appear in these tests. If characteristic precipitates are not obtained, no alkaloid is present.

In making special tests for alkaloids, distribute all the residue upon several small watch-glasses and test each by a reaction more or less specific for a particular alkaloid. If a definite substance is indicated, confirm the first test by other reactions. The "Synopsis of Group II B" (see page 284) is useful in this connection for reference, and also the comparison of the behavior of known alkaloids toward concentrated sulphuric and nitric acid (reagents of Froehde, Marquis, Mandelin and Mecke given on page 237).

An alternative procedure is the following. Dissolve the residue in a little hot alcohol, filter the solution, distribute upon watch-glasses, and evaporate at a gentle heat. The residues may then be tested. Mauch[1] dissolves the residue in 75 per cent. aqueous chloral hydrate solution and uses this solution in testing for alkaloids. The details of this method are given in Chapter V of this book (see page 572).

Purification of the Ether Residue

When the ether residue is contaminated with greasy, resinous, colored or fatty substances, as is often the case in the examination of cadaveric material, many of the tests will either fail entirely or give uncertain results. In this case the residue should be purified in one of two ways.

1. Thoroughly mix the residue with cold water containing hydrochloric acid. Filter to remove insoluble matter (fatty or resinous substances), add sodium hydroxide solution to the filtrate until alkaline and extract with ether. The alkaloids obtained by evaporating the solvent are usually quite pure.

2. This method consists in dissolving the residue in hot amyl alcohol. Extract this solution with a few cc. of very dilute sulphuric

[1] R. Mauch: Contribution at the Congress of the Association of German Druggists, Strassburg 1907.

acid and withdraw the acid solution from the separating funnel. Amyl alcohol will retain greasy and colored impurities, and the alkaloids will be in the aqueous solution as sulphates. Add sodium hydroxide solution in excess and extract with ether. This method of purifying the alkaloidal residue is especially recommended, when considerable coloring matter is present.

Physiological Test.—If the ether extract upon evaporation leaves only a very slight residue, and one that does not taste bitter, frequently no alkaloid is present. The residue consists of fat, resinous matter, or even of traces of some nitrogenous substances, such as peptones (peptide-like bodies) or other cleavage-products of proteins. The examination of cadaveric material by the Stas-Otto process for alkaloids, even after the most painstaking work, frequently produces ether extracts that yield a slight residue when no alkaloid is present. To make perfectly sure as to the presence or absence of alkaloid, dissolve most of the residue in a little water containing hydrochloric acid and test this solution with general alkaloidal reagents. Only when these tests give characteristic precipitates, should the ether residue be examined chemically or physiologically for alkaloid.

In an investigation of more importance, to exclude all possibility of overlooking traces of a particular poison, dissolve a portion of the ether residue in very dilute hydrochloric acid (about 0.5 per cent. HCl). Evaporate this solution upon the water-bath, dissolve the dry residue in 1–2 cc. of water, and inject this solution with a hypodermic syringe into the lymph-sac of a lively frog. Triturate a second portion of the ether residue with a small quantity of flour, or dry bread, and a little sugar,[1] and feed this mixture to a white mouse. If in the course of several hours the experimental animal exhibits no symptoms of poisoning, such as uneasiness, trembling, convulsions, tetanus, diarrhoea, it is quite probable that none of the more powerful alkaloids is present. If a special test has to be made for an alkaloid having mydriatic or myotic properties, the solution of the ether residue prepared as described above should be introduced into one eye of a cat to determine whether or not it causes expansion or contraction of the pupil.

Putrefactive Products of Human Organs and the Stas-Otto Method

Panzer[2] has thoroughly investigated the products that arise as a result of putrefaction of different parts of the human body, particularly with regard to their behavior toward general alkaloidal reagents. The object of this study was to determine whether, in the ordinary procedure of forensic analysis by the Stas-Otto method, ptomaines also may not pass into ether and chloroform extracts, thus making it difficult to detect alkaloids or leading to false conclusions as to the presence of the latter. According to the results of these investigations, the brain alone of all the human organs examined yields substances that give the general reactions of alkaloids even after further purification. Consequently in experiments of this character it is better to exclude the brain entirely, if this

[1] Sugar alone fed to mice easily occasions diarrhoea.

[2] Th. Panzer: Contribution to the Knowledge of Putrefaction of Human Organs. A Contribution to the Stas-Otto Method. Zeitschr. f. analyt. Chem. 47 (1908), 572.

is possible. When the ether extract of the aqueous solution, rendered alkaline with sodium hydroxide, contains amorphous substances that give precipitates with general alkaloidal reagents, these should be removed by dissolving in hydrochloric acid and extracting with ether. This solvent will extract these amorphous substances but not alkaloids. In addition to basic products of putrefaction, Panzer has also discovered acid substances, such as hydro-para-cumaric acid.

CONIINE

Coniine, or α-normal-propyl-piperidine, $C_8H_{16}.NH$, occurs in all parts of spotted hemlock (Conium maculatum) as the dextro-rotatory form, presumably combined with lactic acid. Associated with it are n-methyl-coniine, γ-coniceine and pseudo-conhydrine. It occurs most abundantly (0.2–0.9 per cent.) in not entirely ripe fruit of plants about 2 years old. Leaves and flowers are poorer in alkaloids.

Properties.—Naturally occurring dextro-coniine is a colorless, oily liquid that gradually turns yellow and brown in the air and in more dilute condition has a stupefying odor suggestive of mouse urine. In an atmosphere free from oxygen it boils without decomposition at 166°. Its angle of rotation is $[\alpha]_D = +15.7°$ at 19°. In spite of its high boiling-point, coniine volatilizes in considerable quantity even at ordinary temperature. Coniine is rather difficultly soluble in water at 15° (1:909) but even less soluble in hot water. For this reason a cold, saturated, aqueous coniine solution becomes milky when warmed.

Constitution and Synthesis.—Coniine is a secondary base as shown by its behavior. Heated with acetic anhydride, it forms N-acetyl-coniine:

$$C_8H_{16}.NH + (CH_3.CO)_2O = C_8H_{16}.N.CO.CH_3 + CH_3.COOH,$$

and when shaken with benzoyl chloride and sodium hydroxide solution, it gives N-benzoyl-coniine:

$$C_8H_{16}.NH + C_6H_5.COCl + NaOH = C_8H_{16}.N.CO.C_6H_5 + NaCl + H_2O,$$

and with nitrous acid nitroso-coniine:

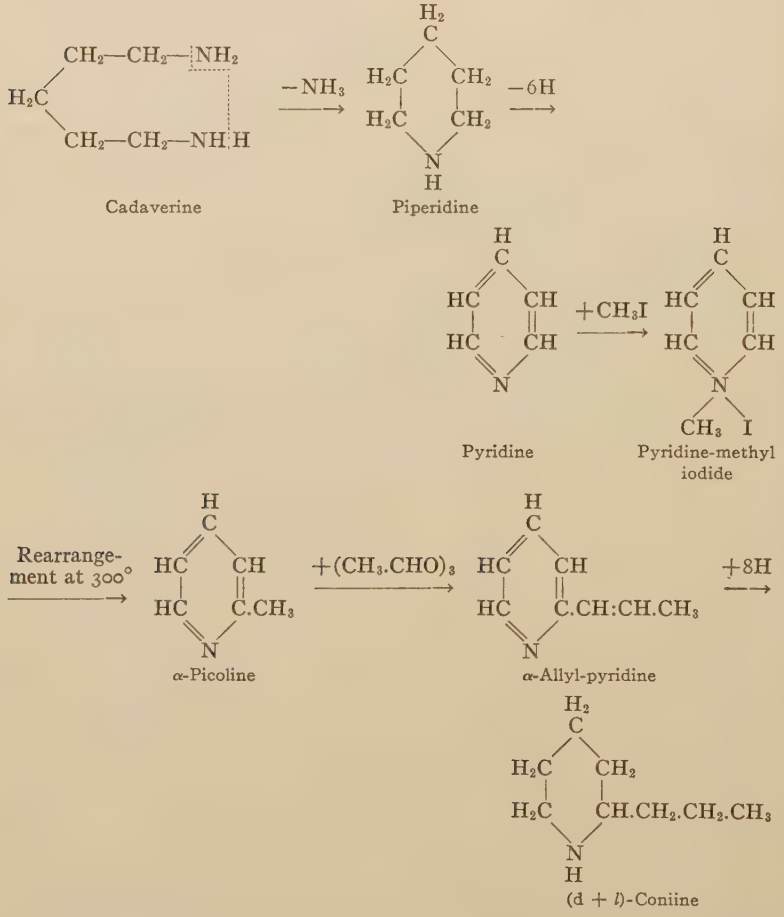
$$C_8H_{16}.NH + HONO = C_8H_{16}.N.NO + H_2O.$$

Finally methyl iodide and ethyl iodide convert coniine into the iodide of methyl and ethyl-coniine:

$$C_8H_{16}.NH + CH_3I = C_8H_{16}.N.CH_3.HI.$$

Coniine may be prepared synthetically by means of the same cadaveric alkaloid, or ptomaine, that is formed in largest quantity in the putrefaction of cadavers, that is, cadaverine or penta-methylene-diamine. If the hydrochloride of this base is heated by itself, piperidine is formed as a result of loss of ammonia and ring-closure. Piperidine, oxidized with silver oxide or heated with concentrated sulphuric acid, loses six atoms of hydrogen and is converted into pyridine. The later as a tertiary base adds methyl iodide and forms pyridine-methyl iodide which at 300° undergoes rearrangement to the isomeric α-picoline hydriodide. Free picoline, obtained by means of potassium hydroxide, condenses when heated

with paraldehyde with loss of water forming α-allylpyridine. Upon reduction with sodium and alcohol, this compound is converted into inactive coniine. If the salt formed by inactive coniine with dextro-tartaric acid is crystallized from water, dextro-coniine tartrate crystallizes out first. When the latter is treated with sodium hydroxide solution, it gives dextro-coniine which is identical with natural dextro-coniine:



Toxic Action.—As a rather strong base, coniine acts locally as a caustic and is therefore easily absorbed even upon external application. Then it exerts a central and peripheral action. The symptoms of intoxication consist of burning in the mouth, itching in the throat, flow of saliva, heaviness in the head, dizziness, nausea, inclination to emesis, tormenting thirst, drowsiness, indistinct vision and hearing, enlarged pupils, weakness in the extremities, great infirmity and cramp of the sural muscles. The principal symptoms, however, consist of

paralysis first of the lower extremities, as a result of which there may be unsteadiness of gait or entire lack of support, then of paralysis of the arms and muscles of respiration. Death ensues from paralysis of respiration. Coniine that is absorbed is eliminated by the lungs and urine. The materials that should be examined are vomitus and stomach contents, blood and organs rich in blood, liver, spleen, lungs, as well as kidneys, and urine. Coniine is said to be fairly resistant to cadaveric putrefaction.

The subject of cadaveric coniine is considered in connection with ptomaines (see page 463).

Detection of Coniine

General alkaloidal reagents especially sensitive to coniine are iodo-potassium iodide (1:8000), phospho-molybdic acid (1:5000), potassium mercuric iodide (1:8000), and potassium bismuthous iodide (1:5000). Coniine picrolonate forms yellow rhombohedrons, melting at 195.5°, and easily soluble in alcohol and warm ether. Gold and platinum chloride fail to precipitate coniine when the concentration is less than 1:100, whereas they precipitate nicotine when the concentration is as low as 1:5000. When coniine is present, the residue from the ether solution has the characteristic odor of this alkaloid. The following tests may be used for its further identification:

1. **Solubility Test.**—Dissolve a drop of coniine in sufficient water to give a clear solution. Gently heat the solution and it will become milky from separation of coniine. A coniine solution that is milky when hot will become clear on cooling. Aqueous coniine solutions have an alkaline reaction. Test the solution with red litmus paper.

2. **Crystallization Test.**—Put a little coniine upon a watch-glass, or glass slide, and add 1–2 drops of hydrochloric acid. Evaporate to dryness and coniine hydrochloride will remain. Immediately after evaporation examine this residue with a microscope magnifying about 200 times. The colorless or faintly yellow crystals are needle-like, or columnar, and frequently are grouped in star-shaped clusters. They show the play of color characteristic of doubly refractive substances.

3. **Melzer's[1] Test.**—Add 2 drops of carbon disulphide and about 2 cc. of alcohol to a trace of coniine and allow it to stand for a few minutes. If 2–3 drops of an aqueous copper sulphate solution (1:2000) are added to this mixture, a distinct yellow to brown color will appear. A similar brownish color is produced by 1–2 drops of very dilute ferric chloride solution even in a coniine solution diluted

[1] H. Melzer: Contributions to Forensic Chemistry. Zeitschr. f. analyt. Chemie 37 (1898), 345.

1:10,000. Dilute beforehand 1 gram of ordinary ferric chloride solution with water to 100 cc. Excess of copper sulphate and ferric chloride solution should be avoided. This reaction is not characteristic of coniine but merely shows the presence of a secondary base. Ether will extract the coloring-matter from coniine solutions rendered brown by copper sulphate, even after dilution with water 1:10,000. Nicotine does not give these colorations.

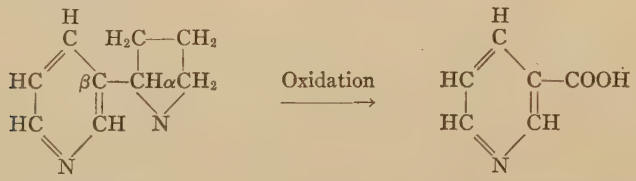
4. Physiological Test.—Subcutaneous injection of at least 15 milligrams of coniine hydrochloride will produce in a frog typical paralysis of the extremities (curare action). Nicotine does not exhibit this action, nor do the cadaveric coniines thus far known.

NICOTINE

Nicotine, $C_{10}H_{14}N_2$, combined with malic and citric acid, occurs in varying quantities (0.5–1.5 per cent.) together with other alkaloids in different species of Nicotiana.

Properties.—Nicotine is a colorless liquid, soon turning yellow and brown upon exposure to air and in time becoming entirely resinous. It has a faint odor of tobacco and a sharp, burning and very persistent taste. In a current of hydrogen it boils without decomposition at 240-242° but during distillation in contact with air undergoes partial decomposition. The free alkaloid is strongly laevorotatory, $[\alpha]_D = -166°$; but its salts are dextro-rotatory. Nicotine is miscible with water in all proportions (distinction from coniine) and freely soluble in alcohol, ether, benzene and petroleum ether. Aqueous and dilute alcoholic nicotine solutions react strongly alkaline to litmus but not to phenol-phthalein.

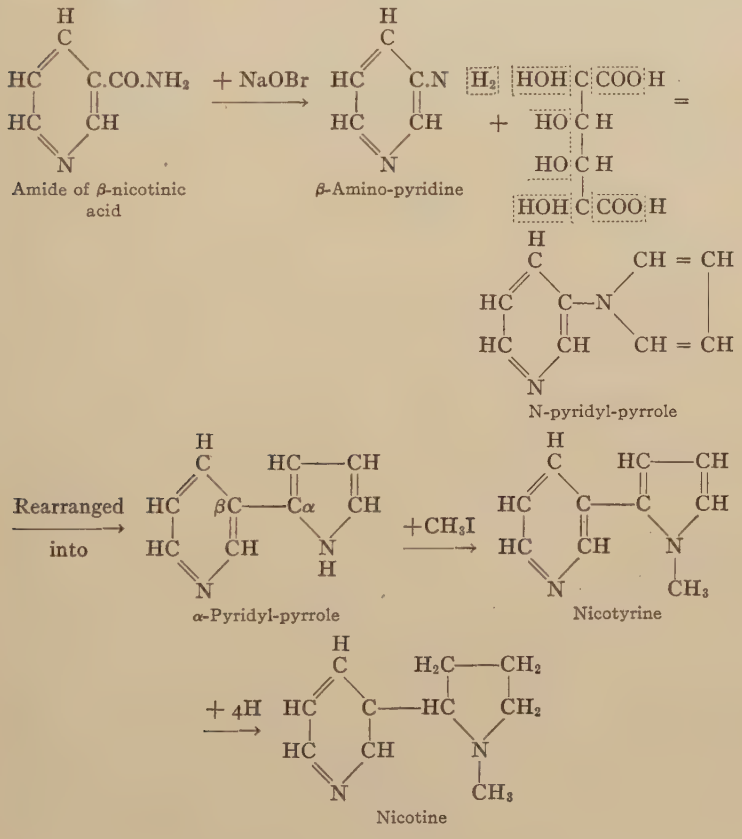
Constitution and Synthesis.—Nicotine is a di-acid base, since it forms well-crystallized salts with one and two equivalents of acid. It combines with 2 molecules of an alkyl iodide, for example, with 2 CH_3I, forming a di-iodomethylate, $C_{10}H_{14}N_2.2CH_3I$, from which moist silver oxide separates nicotine alkyl-hydroxide. By this behavior nicotine shows itself to be a di-tertiary base. Oxidized with nitric acid, chromic acid or potassium permanganate, nicotine is converted into nicotinic acid, or β-carboxy-pyridine. This shows that nicotine is a pyridine derivative having a side-chain in the β-position with respect to the pyridine nitrogen. This behavior is in accord with the structural formula proposed for nicotine by Pinner and substantiated several years later by Pictet's synthesis of nicotine:



Nicotine Nicotinic acid

Pictet, starting from nicotinic acid, effected the synthesis of nicotine. The amide of nicotinic acid obtained in the usual manner was converted, upon treat-

ment with sodium hypobromite according to the method of A. W. Hofmann, into β-amino-pyridine. This compound subjected to dry distillation with mucic acid forms a pyrrole-ring with loss of carbon dioxide and water, giving N- pyridyl-pyrrole. If the vapor of the latter is passed through a gently ignited tube, molecular rearrangement takes place with formation of the isomeric α- pyridyl-pyrrole which forms salt-like compounds with alkali metals. Methyl iodide acting upon the potassium salt forms nicotyrine which differs from nicotine in containing 4 atoms less of hydrogen. For the purpose of rendering the pyrrole-ring of nicotyrine more easily reducible for conversion into nicotine, nicotyrine was converted into a mono-substitution product by means of iodine in alkaline solution. This compound was then reduced to dehydro-nicotyrine by tin and hydrochloric acid. The dibromo-addition product of this compound upon reduction with tin and hydrochloric acid gave tetra-hydro-nicotyrine, or inactive nicotine. This racemic compound converted into its salt with dextro-tartaric acid was resolved into laevo-nicotine, identical with the natural base, and its optical opposite. According to this synthesis, natural nicotine is α-pyridyl-methyl-pyrrolidine:



Toxic Action.—Nicotine is one of the most violent poisons and is hardly inferior to hydrocyanic acid in toxic power and rapidity of action. It probably acts as a poison upon all classes of animals. Absorption of nicotine takes place even in a few seconds from the tongue, the rectum and the eye; somewhat more slowly from the stomach. It is also absorbed from the outer skin. In consequence of this rapidity of absorption, nicotine exhibits its toxic action very quickly, even after a few minutes. Elimination proceeds through the lungs and kidneys. Although not really corrosive in its action, nicotine in more concentrated form, especially as the free alkaloid, acts as a local irritant. After administration of fatal doses by the mouth, usually inflammation of the mucous lining of the stomach does not occur, for the action of nicotine is too rapidly fatal. Moreover, following brief stimulation, nicotine exerts a central paralyzing action upon the brain and spinal cord, and finally as a result of absorption acts upon different organs, such as the heart, eyes, and intestinal tract. In acute nicotine poisoning death ensues from paralysis of the respiratory center, but probably action upon the heart always takes place, although this usually does not lead to death.

The symptoms of nicotine poisoning have been accurately determined by various experimenters who have taken from 1 to 4 milligrams of this alkaloid. There is burning in the mouth and throat, increased secretion of saliva, headache, stupefaction, dizziness, indistinctness of vision and hearing, photophobia, parched throat, coldness of the extremities, emesis and enforced evacuation of the bowels. Respiration was accelerated and labored. At first the pulse increased in frequency but later changed, becoming at irregular intervals more and less frequent. After 45 minutes the experimenters swooned and in one case for 2 hours there were uninterrupted clonic convulsions, especially of the muscles of respiration, with trembling of the extremities and shaking of the whole body. In severer cases the gastric symptoms predominate. The intense diarrhoea that appears may be bloody, resulting in death in a few hours. On the other hand, in severe cases, for example, from tobacco enemas or from nicotine itself, convulsions, delirium and paralysis predominate and may prove fatal in 10–30 minutes.

In suspected nicotine poisoning, the material that should be selected for chemical examination is: urine, blood, stomach and intestines with contents, liver and lungs.

Detection of Nicotine

Ether or low-boiling petroleum ether will extract nicotine from an aqueous alkaline solution. Evaporation of the solvent leaves the alkaloid as an oily liquid having the odor of tobacco and a strong alkaline reaction. Most of the general alkaloidal reagents will precipitate nicotine from quite dilute solutions, in which respect this alkaloid is very different from coniine. Phospho-molybdic acid and potassium bismuthous iodide precipitate nicotine even in a dilutiom of 1:40,000; potassium mercury iodide in 1:15,000; gold chloride in 1:10,000; and platinum chloride in 1:5000. Nicotine

picrolonate forms prismatic needles frequently arranged in clusters and melting at 213°. The following special tests serve for the identification of nicotine:

1. **Crystallization Test.**—Evaporate nicotine dissolved in dilute hydrochloric acid upon a watch-glass. A yellow varnish will remain. Microscopic examination will show it to be entirely amorphous. If kept for a long time in a desiccator over sulphuric acid, it will become indistinctly crystalline. This test is not of as much use in identifying nicotine as in differentiating it from coniine, the hydrochloride of which is characterized by its great tendency to crystallize.

2. **Roussin's Test.**—Dissolve a trace of nicotine in ether, using a dry test-tube. Add to this solution about the same volume of ether containing iodine. Stopper and set the test-tube aside. The mixture will become turbid and deposit a brownish red resin, gradually becoming crystalline. After some time, ruby-red needles having a dark blue reflex will crystallize. These are "Roussin's crystals." If nicotine is old or resinous, as a rule it will not give these crystals.

3. **Melzer's[1] Test.**—If the alcoholic solution of a drop of nicotine is heated with about 2 cc. of epichlorohydrine,[2] the mixture after more or less time, depending upon the concentration, will become distinctly red. The limit of delicacy is 0.25 mg. of nicotine. Under the same conditions coniine gives no color.

4. **Schindelmeiser's[3] Test.**—If 5–10 mg. of non-resinous nicotine is treated first with a drop of formaldehyde solution (about 30 per cent.) free from formic acid and then with a drop of concentrated nitric acid, the mixture takes on a rose-red color. If nicotine and formaldehyde are in contact for several hours, the solid residue

[1] H. Melzer: Contributions to Forensic Chemistry. Zeitschr. f. analyt. Chemie 37 (1898), 345; Reactions of Coniine and Nicotine. Zeitschr. d. allgem. österr. Apotheker-Vereins 54 (1902), 65.

[2] Epichlorohydrine, obtained by the action of potassium hydroxide (1 Mol.) upon α-di-chlorohydrine, $CH_2Cl.CH(OH).CH_2Cl$, or β-dichlorohydrine, $CH_2Cl.CHCl.CH_2.OH$:

$$CH_2Cl.CH(OH).CH_2Cl + KOH = CH_2Cl.\underset{\diagdown\,O\,\diagup}{CH}.CH_2 + KCl + H_2O,$$

Epichlorohydrine

is a colorless liquid insoluble in water and freely soluble in alcohol and ether. It has an odor like chloroform and a burning, sweetish taste.

[3] J. Schindelmeiser: Detection of Nicotine. Pharm. Zentralhalle 40 (1899), 703.

treated with a drop of concentrated nitric acid gives an even finer color reaction, that is, a rose to a dark red color.

Only a little formaldehyde should be used, otherwise the solution becomes green after a while and decomposition occurs. Under the same conditions, trimethylamine, piperidine, pyridine, picoline, quinoline and aniline give no color. Nor do extracts from putrefying horse-flesh and the entrails of animals, poisoned by arsenic or mercury, give the test, at least not when these extracts are prepared according to the Stas-Otto method.

5. Physiological Test of H. Fühner.[1]—For the positive identification of nicotine, the best of all the numerous effects produced by this alkaloid is that upon the central nervous system, for it is exhibited by no other alkaloid related to it toxicologically, chemically or in action. As a result of this action, even a few minutes after injection of nicotine solutions a frog in a sitting posture will draw up its hind legs over the back so that the heels approach, touching when the action is stronger, or the legs even cross over the back. The results observed by Fühner, after injection of 0.2 mg. of nicotine hydrochloride into a frog weighing about 30 grams, were the following: soon after injection respiration ceased; instead of respiratory movements there was trembling and quivering of the flanks; the animal sat motionless, after about 5 minutes drawing up its hind legs over the back. The posture of the front legs is less characteristic than that of the hind legs. They usually lie downward on the belly. After 2–3 hours, respiration and spontaneous movements return. If 1 milligram of nicotine hydrochloride is injected, the typical posture of the hind legs comes to an end in about 30 minutes and they relax. As a result of these and larger doses of nicotine, the muscles of the animal acquire a characteristic condition of stiffness.

This effect upon the frog just described is entirely absent in the case of coniine. A test with pure nicotine is advisable as a check.

ANILINE

Aniline, $C_6H_5.NH_2$, upon evaporation of the ether extract of the aqueous alkaline solution, appears in the form of yellow, reddish or brownish oil-drops. Dissolve some of this residue in water by shaking and apply to the solution the aniline tests already described on page 72. A further test for aniline consists in mixing some of the residue with a few drops of concentrated sulphuric acid and adding a few drops of potassium dichromate solution. If aniline is present, an evanescent blue color will appear.

VERATRINE

Sabadilla seed, the seed of Sabadilla officinalis, contains several alkaloids, namely, cevadine or crystallized veratrine, $C_{32}H_{49}O_9N$,

[1] H. Fühner: Detection and Estimation of Poisons Biologically. E. Abderhalden's Handbuch der Biochemischen Arbeitsmethoden 1911, page 48.

veratridine or water-soluble, amorphous veratrine, $C_{33}H_{49}O_9N$, sabadine and sabadinine, $C_{27}H_{43}O_8N$. The first two bases are the principal constituents of officinal veratrine.

Cevadine.—This base, having the formula $C_{32}H_{49}O_9N.3H_2O$, crystallizes in concentric clusters of needles readily soluble in boiling but only slightly soluble in cold alcohol. These crystals, at first clear and transparent, gradually become milky and opaque from loss of water. Its simple salts do not crystallize well but its gold double salt, $C_{32}H_{49}O_9N.HCl.AuCl_3$, forms yellow needles from hot alcohol and melts at 182°. Cevadine undergoes cleavage in hot alcoholic potassium hydroxide solution into angelic acid,[1] or into its stereo-isomer, tiglic acid, and the base cevine, $C_{27}H_{43}O_8N.3\frac{1}{2}H_2O$, which crystallizes well from dilute alcohol:

$$\underset{\text{Cevadine}}{C_{32}H_{49}O_9N} + H_2O = \underset{\text{Angelic acid}}{C_5H_8O_2} + \underset{\text{Cevine}}{C_{27}H_{43}O_8N}.$$

Since cevadine forms with methyl iodide in ether solution cevadine-methyl iodide, having the composition, $C_{32}H_{49}O_9N.CH_3I$, it is a tertiary base, and since it reacts with benzoic anhydride at 100° forming benzoyl-cevadine, $C_{32}H_{48}O_9N.O.CO.C_6H_5$, and with acetic anhydride forming acetyl-cevadine, $C_{32}H_{48}O_8N.O.CO.CH_3$, its molecule must contain one hydroxyl-group. Crystallized veratrine does not contain methoxyl-groups.

Cevine forms a crystalline potassium compound having the composition, $C_{27}H_{41}O_8NK_2$, which serves for the detection of cevine as well as of crystallized veratrine. If alcoholic potassium hydroxide solution is added to an alkaline solution of cevine, separation of fine needles of this potassium compound begins at once. According to Hess and Mohr,[2] cevine is identical with sabadinine occurring in Sabadilla seed. In addition to the potassium compound, the bisulphate of cevine-sabadinine is especially characteristic. When calculated quantities of cevine-sabadinine and dilute sulphuric acid are brought together, a difficultly soluble crystalline precipitate separates at once. Upon recrystallization from hot water, this compound gives beautifully formed crystals having the composition $C_{27}H_{43}O_8N.H_2SO_4.2\frac{1}{2}H_2O$ and melting at 250°. From 210° on, coloration begins to appear. Upon treatment with benzoyl chloride in presence of sodium hydroxide solution, cevine and sabadinine give one and the same monobenzoyl-derivative.

Cevine is less toxic than cevadine.

According to M. Freund, cevine is converted by hydrogen peroxide into cevine oxide, $C_{27}H_{43}O_9N$, which crystallizes well and contains an additional atom of oxygen. This compound probably belongs to the class of amino-oxides, $R \equiv N{=}O$, for it is easily reconverted into cevine by sulphurous acid.

[1] Angelic acid, or methyl-isocrotonic acid (I), and tiglic acid, or methyl-crotonic acid (II), are stereo-isomeric acids having the following formulae:

I. $H_3C—C—H$ / $H_3C—C—COOH$ (with a double bond between the two C)

II. $H—C—CH_3$ / $H_3C—C—COOH$ (with a double bond between the two C)

[2] K. Hess and H. Mohr: Cevine and Sabadinine. Ber. d. Deutsch. chem. Ges. 52 (1919), 1984.

The method of E. Schmidt may be employed for the separation of crystalline veratrine from water-soluble veratrine in officinal veratrine. Dissolve the officinal preparation in a beaker in strong alcohol. Heat this solution to 60–70° and add enough warm water to produce a permanent turbidity. Cautiously add enough alcohol to clear the solution and allow evaporation to take place slowly at 60–70°. A white, crystalline precipitate will presently appear. Filter with suction, wash the precipitate with a little dilute alcohol, and recrystallize from hot alcohol. This is crystalline veratrine. Clear the filtrate from the crystalline precipitate by adding a little alcohol, and evaporate at 60–70°. This will give a second crop of crystals. By repeating this process several times, one may obtain in a crystalline condition about one-third of the veratrine taken. Finally evaporate the filtrate from the crystalline deposit at the given temperature until there is no longer any odor of alcohol. A considerable quantity of a resinous mass, which is a mixture of both alkaloids, will separate. The aqueous filtrate from this deposit will contain veratridine which may be obtained by rapidly evaporating the solution *in vacuo* over sulphuric acid.

Properties of Officinal Veratrine.—The pure officinal preparation is a white, amorphous powder, appearing crystalline under the microscope. It has a sharp, burning taste, and the minutest quantity introduced into the nostrils excites prolonged sneezing. It is almost insoluble in boiling water and the aqueous extract always has a faintly alkaline reaction. It is quite easily soluble in alcohol (1:4), ether ((1:10), chloroform (1:2), as well as in benzene and amyl alcohol. All these solutions have a strong alkaline reaction. Officinal veratrine melts at 150–155° to a yellow liquid solidifying to a transparent, resinous mass. From its ether solution the alkaloid is usually deposited as a white, amorphous powder. In its reactions officinal veratrine behaves like cevadine and water-soluble, amorphous veratrine, for like these it neutralizes acids completely and the salts formed are as little crystalline as those of pure cevadine. Their solutions upon evaporation give water-soluble, resinous deposits having a bitter and at the same time sharp taste.

Toxic Action.—Veratrine gives rise to external burning and prickling of the skin and mucous membranes, and as a result of this action sneezing, watering of the eyes and roughness of the throat appear. In addition to these effects, internal administration of veratrine occasions the following symptoms: burning extending from the mouth to the stomach, flow of saliva and emesis, colic, bloody, mucous and watery stools, dizziness, delirium, irregular and retarded pulse. The central nervous system is first stimulated (raving, convulsions), then paralyzed. Death ensues following slowing of the pulse from paralysis of the heart and respiratory center. In experiments upon animals Kobert has established elimination of veratrine in the urine. In suspected veratrine poisoning, material suitable for examination is vomitus, gastric and intestinal contents, blood, organs rich in blood, and urine.

Detection of Veratrine

From a faintly acid solution ether extracts very little veratrine; but chloroform and amyl alcohol a considerable quantity. Ether and chloroform extract this alkaloid very readily from an alkaline

solution. The solution of veratrine, prepared with water containing sulphuric acid at a dilution of 1 : 5000, gives precipitates with phosphomolybdic acid, iodo-potassium iodide, tannic acid, potassium mercuric iodide, but shows only a slight turbidity with phospho-tungstic acid and potassium bismuthous iodide. Gold chloride and picric acid barely cause precipitation of veratrine at a dilution of 1 : 1000. The following special tests may be used for recognition of veratrine:

1. **Concentrated Sulphuric Acid Test.**—Pour a few drops of concentrated sulphuric acid upon a trace of veratrine. The alkaloid will have a yellow color and, if stirred, give a yellow solution exhibiting a green-yellow fluorescence. Gradually this color will change to orange, then to blood-red and finally to cherry-red. Gentle heating will hasten this color change and veratrine, dissolved in concentrated sulphuric acid, will give a fine cherry-red solution almost immediately.

Froehde's and Erdmann's reagent give color changes similar to those caused by sulphuric acid.

2. **Concentrated Hydrochloric Acid Test.**—Warm a colorless solution of veratrine, prepared with 1–2 cc. of concentrated hydrochloric acid, for about 10 minutes in a small test-tube in a boiling water-bath. A cherry-red color will appear. This color will persist for several days and be quite distinct even with 0.2 mg. of veratrine.

3. **Concentrated Nitric Acid Test.**—This acid dissolves veratrine with a yellow color but the test is not characteristic.

4. **Weppen's Test.**—Thoroughly triturate 1 part of veratrine with about 5 parts of finely powdered cane-sugar. Stir and add a few drops of concentrated sulphuric acid. The mixture will first become yellow and after some time, beginning at the margin, change to grass-green and later to blue. Breathing upon the mixture will cause the color to change more quickly. Too great an excess of cane-sugar should be avoided.

In this test Laves[1] substitutes an aqueous furfural solution for cane-sugar. Mix 3–4 drops of 1 per cent. aqueous furfural solution with 1 cc. of concentrated sulphuric acid. Add 3–5 drops of this solution to the substance to be tested so that it just touches the edge of the liquid. If veratrine is present, a dark streak will gradually run from the substance into the liquid. At the starting-point it will appear blue or blue-violet and farther away green. If substance and liquid are stirred with a glass rod, the liquid will become dark

[1] E. Laves: Pharm. Ztg. 37, 328.

green. After some time, or more quickly when warmed, the color will become blue and finally violet.

5. **Grandeau's Test.**—Direct addition of 1–2 drops of bromine water to the yellow solution of veratrine in concentrated sulphuric acid produces an immediate purple color almost identical with that appearing when the solution of alkaloid in concentrated sulphuric acid stands for a long time or is gently warmed.

6. **Vitali's Test.**—Dissolve veratrine in a few drops of fuming nitric acid and evaporate the solution to dryness upon the water-bath in a porcelain dish. A yellowish residue will remain. If this is cooled and then moistened with an alcoholic potassium hydroxide solution, the color will change to orange-red or red-violet and stirring will produce a solution having the same color. Atropine, hyoscyamine, scopolamine and strychnine respond to this test in a very similar manner.

7. **Physiological Test.**—Inasmuch as veratrine-like ptomaines have repeatedly been observed, it is often worth while to make a physiological test upon a frog in conjunction with the chemical examination. An aqueous solution of the ether residue should be prepared with the aid of very little acetic acid and injected subcutaneously into rather a large frog. If veratrine is present, movements occurring in the act of vomiting immediately appear. The number of the irregular pulse-beats drops to about half and less, from 60 to 30. With sufficiently large doses, tetanic spasms and symptoms of paralysis predominate from the start. If 0.01 mg. of veratrine as the hydrochloride is injected into the thoracic lymph-sac of a frog, 20 minutes after the injection, according to H. Fühner, distinct symptoms of veratrine-action are in evidence. This is shown by the very stiff and clumsy manner in which the animal takes its first jump, which is brought about only by irritation, whereas the succeeding jumps are soon again normal. This "veratrine-effect" gradually develops and becomes even better. After an hour if the frog sitting at rest is irritated by pinching its toes, it laboriously makes the first sluggish jump after distending and stretching itself. The typical veratrine-effect is best produced by 0.03–0.06 mg. of veratrine hydrochloride. After 0.1 mg. symptoms of central irritation and paralysis appear in conjunction with muscular action. The striking stretching of the hind legs in jumping is even tetanus-like in character. Doses of 0.5–1 mg. are fatal, following which the characteristic veratrine-effect is no longer distinctly in evidence and from the start symptoms of paralysis predominate (H. Fühner).

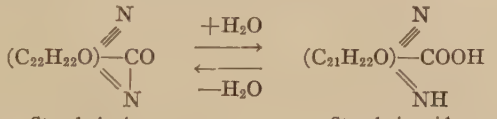
STRYCHNINE

Strychnine, $C_{21}H_{22}O_2N_2$, occurs together with brucine, combined with malic and caffetannic acid, in many Strychnos species. The seeds of Strychnos nux vomica (Nux vomica) contain 0.9–1.9 per cent. of strychnine and 0.7–1.5 per cent. of brucine. Total alkaloids

(strychnine + brucine) amount upon the average to 2.5 per cent. Ignatius beans contain upon the average about 1.5 per cent. of strychnine and about 0.5 per cent. of brucine.

Properties.—The free base strychnine forms colorless, shining prisms belonging to the rhombic system and melting at 268°. The alkaloid dissolves in 6600 parts of cold and 2500 parts of hot water, giving solutions having an alkaline reaction and a very bitter taste. It is nearly insoluble in absolute alcohol and absolute ether; soluble in 160 parts of cold and 12 parts of boiling alcohol (90 per cent. by volume); also soluble in commercial ether and benzene; but most readily soluble in chloroform (6 parts at 15°). Strychnine diluted with water 1:670, 000 can be distinctly recognized by its very bitter taste.

Constitution.—Although strychnine contains 2 atoms of nitrogen in its molecule, it behaves like a monacid base, combining with one equivalent of acid and forming salts that are usually crystalline. These salts have a very bitter taste. The tertiary nature of this base is shown by the fact that strychnine combines with methyl iodide at 100°, forming strychnine iodo-methylate, $C_{21}H_{22}O_2N_2.CH_3I$, which with moist silver oxide forms an ammonium-base, the easily soluble strychnine methyl-ammonium hydroxide, $C_{21}H_{22}O_2N_2.CH_3.OH$. If strychnine is heated with sodium methylate in alcoholic solution and then acidified with acetic acid, it yields microscopic crystals of strychnic acid. This behavior indicates that strychnine is probably the inner anhydride of strychnic acid and contains a group of the character of an acid amide (Tafel[1]):



Strychnine[1] Strychnic acid

As a result of the action of hydrogen peroxide upon strychnine, strychnine oxide, crystallizing in colorless prisms, is formed together with other products of oxidation. This compound according to its properties belongs to the class of amino-oxides and, upon the basis of Tafel's strychnine formula, has the formula, $\begin{matrix} OC \\ N \end{matrix}\!\!>\!(C_{20}H_{22}O) \equiv N:O$.[2] When strychnine is heated with water at 160–180°, it is converted into the isomeric iso-strychnine[3] which has an action similar to that of curare. Strychnine, brucine, iso-strychnine and curare form a continuous series in which from the first to the last member the power of producing convulsions diminishes, whereas the action causing paralysis of the motor nerve-endings ("curare-action") increases.

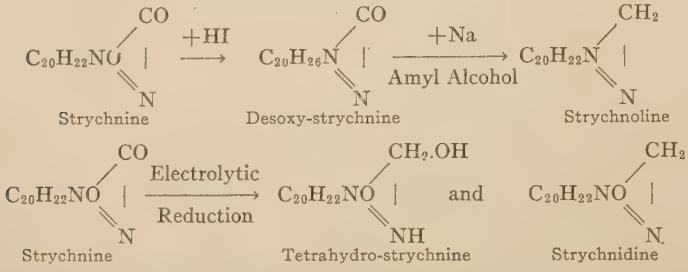
Reduction of strychnine by means of hydriodic acid converts it into desoxy-strychnine, $C_{21}H_{26}ON_2$, from which by treatment with sodium in boiling amyl

[1] J. Tafel: Strychnine. Annalen d. Chemie 264 (1891), 35.

[2] A. Pictet and Mattisson: Strychnine Oxide. Ber. d. Deutsch. chem. Ges. 38 (1905), 2782.

[3] Bacavescu and A. Pictet: Iso-strychnine. Ber. d. Deutsch. chem. Ges. 38 (1905), 2787.

alcohol solution is obtained the acid-free compound strychnoline, $C_{21}H_{26}N_2$. Electrolytic reduction of strychnine in strong sulphuric acid solution gives tetrahydro-strychnine, $C_{21}H_{26}N_2O_2$, and strychnidine, $C_{21}H_{24}ON_2$. These two bases may be separated by warm water, in which tetrahydro-strychnine is soluble and strychnidine nearly insoluble. Tetrahydro-strychnine and strychnidine are powerful convulsive poisons. According to Tafel, these reduction products of strychnine have the following formulae:



Physiological Action.—Strychnine increases reflex irritability of the brain and spinal cord. Even the slightest stimulus, especially acoustic, optical, or tactile, may cause powerful reflexes after large doses of this alkaloid. Convulsions may follow each stimulus, if the dose is large enough. Very large doses of strychnine cause curare-like paralysis of the ends of motor nerves in frogs and warm-blooded animals. It may also affect the muscles of the heart. Strychnine diminishes the motile power of leucocytes and then arrests their motion. The poison also affects plant protoplasm, at least that of Mimosa pudica, in that the plant's motor organs lose their elacity and flexibility.

The symptoms of strychnine intoxication, appearing after varying lengths of time but usually after 15–30 minutes, consist of stiffness of the neck, stiffness, lock-jaw, frothing at the mouth, cessation of respiration, and especially tetanus which appears in repeated attacks every 10–15 minutes. The eyeballs protrude from their sockets during these attacks and the pupils are enlarged. There is severe cyanosis but this disappears as the attack subsides. Emesis rarely occurs. Sometimes the attacks appear to be spontaneous, at other times they are the result of external stimuli, noises, slight movements, flashes of light from a reflector. Individual attacks last 3–4 minutes and death usually occurs at the third or fourth attack, within a period of 10 minutes to 3 hours, as the result of asphyxiation. The rapidity with which *rigor mortis* appears is quite noticeable.

Behavior of Strychnine in the Animal Body and Elimination

Absorption of strychnine from the intestinal tract probably proceeds rather slowly, since in cases of poisoning most of this alkaloid, is found in the stomach and sometimes also in the intestines. According to Bakunin and Majone,[1] the amount of strychnine found in the organs of animals is usually very small and rarely exceeds a tenth of the quantity administered. In dogs elimination of

[1] W. Bakunin and V. Majone: Toxicological Experiments with Strychnine. Gaz. chim. ital. 36 (1905), II, 227.

strychnine in the urine is at an end 72 hours after administration. According to Salant,[1] strychnine undergoes in the intestines of animals changes of such a character that it can no longer be recognized as such by chemical tests, whereas even 1 mg. of the alkaloid in the stomach of the same animals can be distinctly recognized. From the results of these experiments it follows that stomach and intestines should be examined separately, if it is a matter in legal cases of testing for strychnine in the cadaver. A. de Dominicis[2] maintains as the result of experiments upon animals that strychnine may also be detected in the bones of animals poisoned by this alkaloid, even when the quantities of the poison are small. The bones examined were carefully freed from all soft parts, then finely ground, and tested for strychnine by the Stas-Otto method. Elimination of strychnine from the organism, aside from saliva, bile and milk, takes place mainly in the urine, and in the case of man unaltered in form. Elimination begins even in the first hour, is slight after 2 days, and ceases entirely much later. In small doses the percentage of strychnine eliminated unchanged in the urine is less than after larger doses, in which case 70–75 per cent. of it remains undestroyed. Strychnine can be stored up unchanged in the liver, kidneys, brain and spinal cord (see above).

According to experiments made by Hatcher and Eggleston[3] upon dogs, cats and guinea-pigs, toxic but not lethal doses of strychnine, amounting in all to 25 times that of a single lethal dose, may be administered at short intervals in the course of 12 days without lasting ill-effects. Only a small part of the strychnine administered could be found again in the urine; elimination through this channel ceased within 24–48 hours. Strychnine could not be detected in the faeces. A very large part of the strychnine taken was rapidly destroyed in the animal organism. Only when death ensued within 3 hours after the last dose of strychnine, was it possible to find this alkaloid in the tissues.

The stability of strychnine in the cadaver seems to be as certain as the rapidity with which most of it is destroyed in the living organism as a result of animal metabolism, for Cram and Meserve[4] found unchanged strychnine in the liver and spinal cord of the cadaver of a man exhumed after 4 months. Kratter was able to detect it positively even after 6 years. Consequently strychnine sometimes resists putrefaction in cadavers for quite a long time.

Cadaveric Strychnine.—Mecke[5] isolated from a cadaver, that had already begun to putrefy, a crystalline ptomaine very much like strychnine in its chemical behavior. This ptomaine, however, differed from strychnine in having only a faintly bitter taste, in being non-toxic, and in exerting no tetanizing action upon frogs.

[1] W. Salant: Zentralbl. f. inn. Med. 23, 902.

[2] A. de Dominicis: Detection of Strychnine in Bones. Vierteljahrsschr. f. gerichtl. Med. u. Sanitätsw. 28 (1904), 284.

[3] R. A. Hatcher and C. Eggleston: Fate of Strychnine in the Body. Journ. Pharymacolog. and Exper. Therapeutics 10 (1917), 281.

[4] M. P. Cram and P. W. Meserve: Stability of Strychnine in the Cadaver. Journ. of Biol. Chem. 8 (1910), 495.

[5] Mecke and Wimmer: A Cadaveric Alkaloid resembling Strychnine. Pharm. Ztg. 43 (1898), 300.

Detection of Strychnine

Potassium and sodium hydroxide, ammonia and alkali carbonates precipitate the free strychnine base from aqueous solutions of its salts as a white, crystalline solid:

$$C_{21}H_{22}N_2O_2.HNO_3 + NaOH = C_{21}H_{22}N_2O_2 + H_2O + NaNO_3.$$

Ether will extract strychnine from an alkaline solution and deposit the alkaloid upon evaporation in fine crystalline needles. Chloroform takes up the alkaloid more freely, since strychnine is considerably more soluble in this solvent than in ether. Even very dilute solutions of strychnine salts give precipitates with most of the alkaloidal reagents. Tannic acid, potassium mercuric iodide and phospho-tungstic acid produce white precipitates; gold chloride and phospho-molybdic acid yellow precipitates; and iodo-potassium iodide a brown precipitate. To obtain tests with these reagents, the residue from ether should first be dissolved in very dilute hydrochloric acid.

Concentrated sulphuric acid, Erdmann's and Froehde's reagent dissolve perfectly pure, brucine-free strychnine without color.

Concentrated nitric acid dissolves strychnine with a yellowish color. Potassium dichromate, added to aqueous solutions of strychnine salts, precipitates strychnine dichromate, $(C_{21}H_{22}N_2O_2)_2.H_2Cr_2O_7$, in the form of fine, yellow, crystalline needles which upon recrystallization from hot water appear as shining orange-yellow needles.

Potassium ferricyanide, added to solutions of strychnine salts, precipitates golden yellow, crystalline strychnine ferricyanide, $(C_{21}H_{22}N_2O_2)_2.H_3Fe(CN)_6.6H_2O$.

Special Reactions

1. **Sulphuric Acid-Dichromate Test.**—Dissolve a trace of strychnine in 2–3 drops of concentrated sulphuric acid upon a watch-glass. Add a particle of potassium dichromate and hold it firmly in one place upon the glass. Intense blue or blue-violet streaks will come from the potassium dichromate, if the watch-glass is tilted up and down. If the entire mixture is stirred, the sulphuric acid will have a beautiful evanescent blue or blue-violet color.

This test may also be made by scattering upon the surface of the solution of strychnine in concentrated sulphuric acid a few particles

of coarsely powdered potassium dichromate and mixing well with a glass rod. In this way the blue to blue-violet color-reaction is given very well. The blue color is not permanent. It soon changes to red and finally to dirty green.[1]

Strychnine dichromate and ferricyanide give this test especially well. To convert the residue from the ether extract of the aqueous alkaline solution into the former salt, pour a very dilute potassium dichromate solution over the residue. When the two substances have been in contact for some time, pour off the remaining liquid. Wash the product once with a little water thoroughly draining off the water. Put some of the residue upon the end of a glass rod and draw it through a few drops of concentrated sulphuric acid upon a watch-glass. If strychnine is present, blue and violet streaks will appear. The test may also be made by moistening the residue direct with a few drops of concentrated sulphuric acid.

Other oxidizing agents may be substituted for potassium dichromate, such as potassium permanganate, lead peroxide, manganese dioxide, potassium ferricyanide, cerium oxide and vanadic acid. But neither potassium nitrate nor nitric acid can be used, as these reagents even prevent this test. Consequently strychnine nitrate does not give the test.

Mandelin's reagent,[2] that is, vanadic-sulphuric acid, gives this strychnine test very well. The blue or violet color given by this reagent with strychnine is more permanent than that produced by potassium dichromate. The color finally changes to orange-red.

Sonnenschein's reagent[2] dissolves strychnine with a blue color gradually changing to red.

2. Wharton's[3] Test.—Dissolve the substance to be tested in a dry condition in chloroform. Put this solution in a small test-tube and evaporate the chloroform by setting the tube in a larger one containing boiling hot water. When the substance is dry or nearly so, add a few drops of a mixture of equal parts of strong sulphuric acid and water and dissolve by shaking. Now introduce bromine tapor carefully and move the tube to and fro so that the solution vakes up bromine. Replace the tube in boiling water to expel excess of bromine vapor. If strychnine is present, a carmine-red color

[1] According to Tafel (Ann. d. Chemie 268 (1892), 233), this color-reaction is characteristic of many anilides and is due to the presence of the group —CO—N=.

[2] See "The Preparation of Reagents" page 640.

[3] J. C. Wharton: Test for Strychnine with Bromine. Journ. Pharm. 8 (1901), 201.

will appear in a few minutes, increasing in intensity as the bromine evaporates. This color fades after a time. Instead of bromine vapor, a solution of a drop of bromine in 2 cc. of chloroform may be used. If the quantity of strychnine present is small, only a little bromine should be added to the solution.

3. **Physiological Test.**—Dissolve the ether residue in a few cc. of very dilute hydrochloric acid. Evaporate the filtered solution to dryness upon the water-bath. Dissolve the residue in pure water (about 1 cc.) and inject the solution into the lymph-sac of a lively frog. Then put the frog in a large, loosely covered beaker. Toxic symptoms will appear in 5–30 minutes, depending upon the quantity of strychnine. This alkaloid does not increase reflex irritability for all kinds of stimuli but only for tactile, optical and especially for acoustic stimuli. When the dose of strychnine is sufficiently large, each kind of stimulus mentioned will produce convulsions like those caused by tetanus. For example, if the beaker containing the "strychnine-frog" is gently tapped, this slight acoustic stimulus is sufficient to produce convulsions. The convulsions produced in a frog by strychnine, contrary to those caused by picrotoxin, are characterized by a prevailing extended position of the hind legs. According to H. Führer, between 0.02–0.05 mg. of strychnine nitrate is the smallest quantity capable of producing tetanic convulsions in a medium-sized frog. With 0.05 milligram the animals show increased reflex irritability, the first tetanic attacks following after 20–30 minutes upon external stimulus, especially acoustic. At first they are of short duration, as the animals again draw up their hind legs after the convulsion. Later the legs are kept continuously extended and convulsions occur without external stimulus. During these convulsions the front legs are crossed. With doses of about 1 mg. of strychnine nitrate paralysis, in conjunction with irritability and following close upon it, begins to predominate but this is striking only in water-frogs.

Detection of Strychnine in Presence of Brucine

More than traces of brucine may entirely prevent or interfere with the detection of strychnine with concentrated sulphuric acid and potassium dichromate. Sometimes Mandelin's reagent will show strychnine in presence of brucine. For the more certain detection of strychnine, dissolve the ether residue containing brucine in about 2 cc. of dilute sulphuric acid, add 2 drops of concentrated nitric acid, and allow the mixture to stand 3–4 hours in the cold. Render strongly alkaline with sodium hydroxide solution and extract thoroughly with ether. The residue from ether will be brucine-free or nearly so. In case the material contains strychnine, it will give very satisfactory tests with concentrated sulphuric acid and potassium dichromate, as well as with Mandelin's and Sonnenschein's reagent. Under the experimental conditions described, the easily oxidized brucine, but not the more resistant

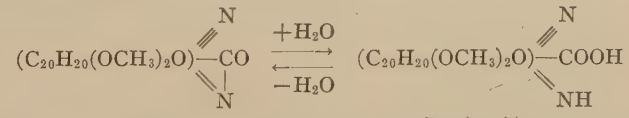
strychnine, is destroyed by oxidation. Extraction of the alkaline solution with ether removes strychnine but not the oxidation products of brucine.

BRUCINE

Brucine, $C_{23}H_{26}N_2O_4.4H_2O$, always occurs together with strychnine in the seeds of Strychnos nux vomica and in Ignatius beans. The bark of Strychnos species, however, appears to contain together with brucine only traces of strychnine.

Properties.—Brucine crystallizes upon evaporation of its dilute alcoholic solution in colorless, transparent, monoclinic plates with 4 molecules of water of crystallization. Brucine loses part of its crystal water in dry air and all *in vacuo* over sulphuric acid, as well as when heated at 100°. From strong alcohol it crystallizes with 2 molecules of crystal water. This hydrated brucine melts somewhat above 100° in its crystal water. The anhydrous alkaloid melts at 178°. Brucine belongs to those alkaloids that are relatively easily soluble in water. Crystallized brucine dissolves in 320 parts of cold and 150 parts of boiling water, giving an alkaline, laevo-rotatory solution having a very bitter taste. This alkaloid is easily soluble in alcohol (1:2), and also in acetone and chloroform; in absolute ether on the other hand it is almost insoluble.

Constitution.—Brucine is a rather strong, monacid base, forming salts with one equivalent of acid that usually are crystalline. Caustic alkalies and alkaline carbonates precipitate brucine from aqueous solutions of these salts. Ammonia in excess first exerts a solvent action upon brucine but gradually it separates from solution in crystalline form. Brucine nitrate, $C_{23}H_{26}N_2O_4.HNO_3.2H_2O$, crystallizes in prisms. Since brucine combines with methyl iodide, forming brucine iodo-methylate, $C_{23}H_{26}N_2O_4.CH_3I$, the tertiary nature of this alkaloid is shown. By means of Zeisel's method 2 molecules of methyl iodide can be obtained with hydriodic acid. Consequently brucine contains two methoxyl-groups. Brucine, heated at 80° with a solution of sodium in absolute alcohol, and also with alcoholic sodium hydroxide, yields upon acidification with acetic acid brucic acid, $C_{23}H_{28}N_2O_5.H_2O$, the molecule of which has been formed by the addition of a molecule of water. Since this acid forms a nitrosamine with nitrous acid, one imino-group (NH) must be present. Tafel and Moufang[1] express the relationship between brucine and brucic acid as follows:



Brucine Brucic acid

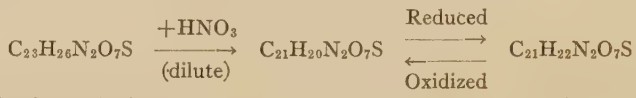
Heated with water, brucic acid is converted into brucine. Consequently brucic acid is related to brucine as strychnic acid is to strychnine.

Hydrogen peroxide converts brucine into the beautifully crystalline brucine oxide, $C_{20}H_{20}(OCH_3)_2N(CO.N:):N:O$, which is reduced by sulphurous acid to brucine. Brucine oxide therefore belongs to the class of amino-oxides.

[1] N. Moufang and J. Tafel: Brucine. Ann. d. Chem. 304 (1898), 24.

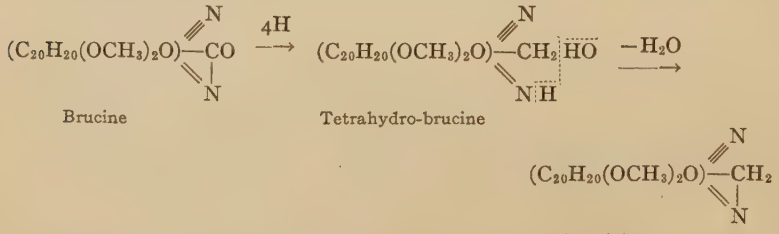
Action of Nitric Acid.—When brucine is boiled with 5 per cent. nitric acid and the product of the reaction is decomposed with sodium acetate, nitro-brucine hydrate, $C_{21}H_{21}(NO_2)(NCH_3)_2N_2O_5$, crystallizing in gold-yellow leaflets is formed. By the action of concentrated nitric acid (sp. gr. 1.4 = 61 per cent.) brucine is decomposed with cleavage of 2 molecules of methyl nitrite, $CH_3.ONO$, which breaks into NO and CO_2. Brucine gives the so-called cacoteline, $C_{21}H_{21}(OH)_2(NO_2)N_2O_3.HNO_3$, which is precipitated by water orange-yellow and can be recrystallized from water containing nitric acid. It is also formed by warming brucine with 20 times the quantity of 20 per cent. nitric acid at 60–70°. Cacoteline is the nitrate of bi-desmethyl-nitro-brucine hydrate, $C_{21}H_{21}(OH)_2(NO_2)N_2O_3$, obtained by dissolving cacoteline in sodium hydroxide solution and neutralizing with acetic acid. The probable explanation of the conversion of brucine into cacoteline is that the two methoxyl-groups of brucine give rise to quinone-formation:[1]

Isolation of the quinone is more easily accomplished by starting with brucine sulphonic acid than with brucine itself. Like all quinones it is reduced by sulphurous acid to the corresponding colorless quinol which is converted back into quinone by cold dilute nitric acid (Leuchs and Geiger):



Brucine sulphonic acid — Quinone — Quinol

Reduction.—Electrolytic reduction in sulphuric acid solution converts brucine into tetrahydro-brucine which loses water when heated in a current of hydrogen and yields brucidine:



Brucine — Tetrahydro-brucine — Brucidine

Physiological Action.—The power of brucine to give rise to tetanic convulsions is about 40–50 times less than that of strychnine. Its curare-like action, however, is stronger than that of strychnine. Following internal administration, elimination of brucine runs parallel with absorption so that cumulative action is not to be feared as in case of strychnine.

[1] H. Leuchs and R. Anderson: Contribution to the Knowledge of Brucine. The Nitric Acid Reaction. (Strychnos Alkaloids XI.) Ber. d. Deutsch. chem. Ges. 44 (1911), 2136.

Detection of Brucine

Ether, benzene or chloroform will extract brucine from an aqueous alkaline solution. Owing to its slight solubility, several extractions with ether are necessary. Evaporation of the ether extract usually leaves the alkaloid in an amorphous condition. The sensitiveness of the alkaloidal reagents toward brucine is as follows: iodo-potassium iodide (1:500,000); potassium mercuric iodide (1:30,000); gold chloride (1:20,000); potassium bismuthous iodide (1:5000); tannic acid and phospho-molybdic acid (1:2000); and platinic chloride (1:1000). Brucine picrolonate forms cubic or rhombic crystals that darken at 254° and melt at 256°. The following tests may be employed for the more certain recognition of brucine:

1. **Nitric Acid-Stannous Chloride Test.**—Concentrated nitric acid dissolves brucine and its salts with a blood-red color, at once changing to red-yellow and finally to pure yellow. Add a few drops of freshly prepared, dilute stannous chloride solution to this yellow-red or yellow solution in a test-tube. A fine violet color will appear. If the solution is now warmed and a drop of nitric acid added, usually the red-yellow color reappears and further addition of stannous chloride solution restores the splendid violet color. The smaller the quantity of nitric acid, the more likelihood that this test will give a good result.

2. **R. Mauch's Modification of Nitric Acid-Stannous Chloride Test.**—An excellent result may be obtained with this test in the following manner. Dissolve brucine in 60 per cent. aqueous chloral hydrate solution and put about 0.5 cc. of this solution into a test-tube. Add very little dilute nitric acid and thoroughly mix the two solutions. Add this mixture to 3 times its volume of concentrated sulphuric acid so that the former is on the surface. A yellow-red to deep red zone will appear immediately. When the upper layer becomes yellow, introduce from a pipette a little dilute stannous chloride solution as an upper layer. A fine violet zone will appear between the two upper layers. The intensity of this color will gradually increase, especially if the test-tube is gently tilted to and fro. Prepare the stannous chloride solution by dissolving 1 part of stannous chloride, $SnCl_2.2H_2O$, in 9 parts of hydrochloric acid having a specific gravity of 1.12 (about 24 per cent. HCl).

ATROPINE

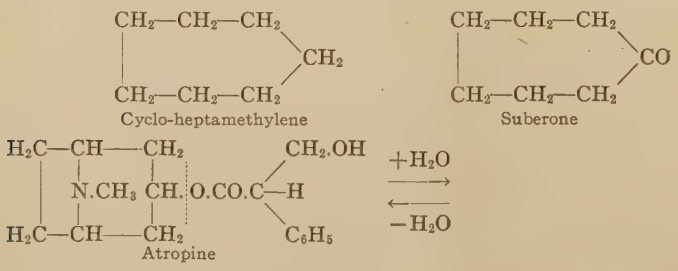
The belladonna plant, Atropa Belladonna, contains alkaloids in its various parts. These consist almost exclusively of hyoscyamine,

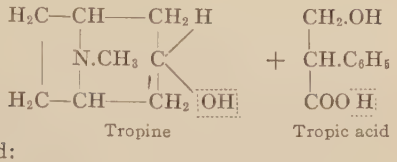
that is, in the leaves up to 0.4 per cent., in the unripe, dried fruit 0.8 per cent. (ripe 0.4 per cent.), and in the root 0.5 per cent. of alkaloid. The thorn-apple, Datura Stramonium, contains hyoscyamine and not atropine. Atropine obtained from the different parts of the plant is formed, at least most of it, as a result of molecular rearrangement, that is, by conversion of hyoscyamine into its racemic form.

Properties.—Atropine, $C_{17}H_{23}NO_3$, crystallizes in shining pointed needles, melting at 115° and dissolving in 600 parts of water, 50 parts of ether, and 3.5 parts of chloroform. It is also soluble in alcohol, amyl alcohol and benzene. The aqueous solution of the alkaloid is alkaline and has a persistent, unpleasant, bitter taste. Unlike the optically active hyoscyamine, atropine is inactive. Atropine is volatile in small quantity with steam.

Constitution.—Atropine is a strong, monacid base, neutralizing acids with formation of salts that do not always crystallize well. A fact to be noted is that aqueous solutions of atropine salts in time undergo decomposition. Atropine sulphate, used in medicine, has the formula, $(C_{17}H_{23}NO_3)_2H_2SO_4.H_2O$, since atropine, heated in ether-alcohol solution in sealed tube at 100° with ethyl iodide, gives atropine iodo-ethylate, $C_{17}H_{23}NO_3.C_2H_5I$, from which silver oxide sets free atropine-ethyl hydroxide, $C_{17}H_{23}NO_3.C_2H_5.OH$, the tertiary character of this alkaloid is shown. From the fact that atropine gives, together with other substances, methyl amine, $CH_3.NH_2$, when heated with soda lime, a methyl-group must be attached to nitrogen, that is, the molecule contains the $N.CH_3$ group. Moreover atropine is an ester, for it is hydrolyzed by fuming hydrochloric acid, slowly at ordinary temperature but rapidly and completely at 100–130°, into tropine which is both a secondary alcohol and tertiary base, and tropic acid. A portion of the tropic acid first formed loses water forming atropic acid. Longer boiling with barium hydroxide solution will also bring about this reaction.

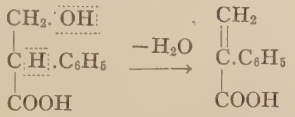
Atropine may be regarded as a derivative of cyclo-heptamethylene, for it can be made to give suberone. Starting from the latter, R. Willstätter (1901) synthesized tropine and thus was able to synthesize atropine itself. Since the constitution of tropic acid is also known as the result of synthesis, that of atropine is established beyond question. To reverse the process of hydrolysis, or saponification of the ester atropine, and re-form the ester, it is necessary to heat tropic acid and tropine for rather a long time upon the water-bath with 20 times the quantity of 5 per cent. hydrochloric acid. The following formulae serve to illustrate the reactions mentioned above:





Tropine Tropic acid

Conversion of tropic into atropic acid:



Tropic acid Atropic acid

Hyoscyamine is stereo-isomeric with atropine. Optically active hyoscyamine, $[\alpha]_D = -20.97°$, is changed into inactive atropine, when heated at 110° with exclusion of air, or when its alcoholic solution is allowed to stand in the cold with a few drops of sodium hydroxide solution until it has become optically inactive.

Physiological Action.—Atropine produces paralysis in different peripheral organs. Moreover it acts upon the central nervous system, especially in larger doses, increasing the irritability of the brain, that is, it first causes irritability and then paralysis. Among the peripheral organs upon which atropine acts is the eye, in which it causes enlargement of the pupils and destroys the possibility of accommodation for objects close at hand. Enlargement of pupils after atropine has been dropped into the eye is most marked in human beings and cats and less in rabbits and dogs. In addition the secretion of almost all glands is suppressed by atropine or at least very much diminished. Stimulation of an animal treated with atropine elicits no secretion of saliva from the submaxillary gland. Under the influence of atropine, secretion of sweat and mucus ceases. The pancreatic secretion increased by muscarine is suppressed by atropine, that of the bile diminished. Upon this property of depressing glandular secretions is based the therapeutic application of atropine for night-sweats and symptoms of poisoning. Since atropine like hyoscyamine is easily absorbed, the first symptoms of intoxication usually appear in a few minutes. Among the symptoms that manifest themselves are: dryness of the mouth and throat, hoarseness, difficulty in swallowing, or it becomes wholly impossible, bright redness and swelling of the face, quickening of the pulse, skin dry, scarlet-red and hot. Further there is bulging of the eye-balls, enlargement and immovability of the pupils, in conjunction with many disturbances of vision, such as weakness of sight and loss of accommodation for objects near at hand. Still other symptoms mentioned by Kobert are: Headache, dizziness, hallucinations, delirium and raving, manifesting themselves in running, dancing, foolish talk, laughter and falling down. These severe symptoms of irritation do not appear until a later stage of poisoning. In case of poisoning by hyoscyamine free from atropine this stage of irritation is almost entirely lacking. The typical mydriatic action of atropine and hyoscyamine is not noticeably evident in the case of their two cleavage-products, tropine and tropic acid. Both mydriatics are easily absorbed and within a comparatively short time are eliminated for the most part unchanged in the urine. On several occasions in poisonings that did not end fatally, Kratter was able to get a distinct

test for atropine in urine. Unquestionably a portion of the atropine absorbed is destroyed in the organism. Experiments made by Gonnermann,[1] showing that the liver, pancreatin and trypsin cause cleavage of atropine, are in favor of this view. Pepsin had no action, nor bacterium coli communis and the contents of human intestines.

Stability of Atropine during Cadaveric Putrefaction

Atropine, whether taken as the pure alkaloid or in the form of deadly nightshade, is rapidly absorbed from all parts of the body and disseminated in the body by the blood-stream in proportion to the distribution of the blood. Elimination begins quickly and takes place through all the channels of elimination of the body. In man and animals, as a result of its specific action in paralyzing secretions, elimination of atropine extends over a longer period, lasting 4–5 days in man after poisoning from 3–5 Atropa berries, and in a dog 14 days after subcutaneous injection of 0.5 gram of atropine sulphate (Ipsen[2]). After very thorough investigations, contrary to the observations and views of other analysts, Ipsen came to the conclusion that atropine can still be detected in parts of cadavers after a rather long time. He succeeded in detecting this alkaloid even after 12 years when a quantity of 0.03 gram of sulphate in 300 cc. each of blood, urine and beer, or of pure alkaloid in 300 cc. of blood, was exposed to the effects of decomposition at 35°. Atropine therefore is extraordinarily resistant to putrefaction. In the viscera of a cadaver exhumed after 3 years, Ipsen detected atropine in quantities that could be estimated, and identified it as such with certainty by tests upon the human eye.

Material suitable for examination is gastric and intestinal contents, organs rich in blood, blood and especially urine. When poisoning has been caused by parts of the plant, search for them should be made in the gastric and intestinal contents. Even after they have left the intestines, seeds still retain a portion of the alkaloid having mydriatic action. According to Ipsen, one such seed is said to be sufficient, after it has been cleaned, ground and extracted with very slightly acidulated water, to give a solution that will produce the characteristic enlargement of the pupil when introduced into the eye.

Detection of Atropine

Ether, benzene or chloroform will extract atropine from an aqueous solution rendered alkaline with sodium hydroxide. In a special search for atropine, homatropine or scopolamine, when morphine need not be considered, render the aqueous tartaric acid solution alkaline with sodium carbonate, or better bicarbonate, and extract with chloroform or ether, since sodium hydroxide may saponify these mydriatic alkaloids which are esters. Upon evaporation of

[1] M. Gonnermann: Capacity of Liver Histozym and Some Enzymes to Cause Cleavage of Some Glucosides and Alkaloids. Pflüger's Archiv 113 (1906), 168.

[2] C. Ipsen: Detection of Atropine. Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw. 31 (1906), 308.

the ether solution, atropine usually appears as a non-crystalline varnish. Even very dilute solutions of atropine and hyoscyamine give precipitates with most of the general alkaloidal reagents. R. Eder[1] has tested the sensitiveness of these reagents to atropine and hyoscyamine, dissolved in 0.5 n-sulphuric acid. Precipitation was made upon watch-glasses and 0.5 cc. of the alkaloid solution and 2 drops of the reagent were used for each test. The period of observation was 2–3 minutes. Atropine and hyoscyamine showed the same limits of precipitation where turbidity or faint precipitation could just be seen, that is: iodo-potassium iodide 1:200,000; phospho-tungstic acid: 1:100,000; potassium bismuthous iodide 1:100,000; phospho-molybdic acid 1:70,000; potassium mercuric iodide 1:10,000; silico-tungstic acid 1:20,000; gold chloride 1:1000. Iodo-potassium iodide produces in aqueous solutions of atropine salts a red-brown precipitate that changes within 5–10 minutes into blue-green, shining, metallic leaflets of the hydriodide of atropine-tetriodide, $C_{17}H_{23}NO_3$-I_4.HI. This is characteristic of atropine, especially upon microscopic examination.

Special Reactions

1. Vitali's[2] Test.—Evaporate upon the water-bath in a porcelain dish atropine or an atropine salt with a few drops of fuming nitric acid. Moisten the yellowish residue when cold with a few drops of a solution of potassium hydroxide in absolute alcohol (about 1:10). An evanescent violet color at once changing to a fine red will appear.

In addition to atropine, hyoscyamine, scopolamine and eumydrine[3] also give Vitali's test. Pseudo-aconitine gives with nitric acid a yellow residue that becomes purple-red when moistened with alcoholic potassium hydroxide. Vitali has further shown that the succeeding red color is not characteristic, since strychnine gives a fine red or a transitory violet color. Veratrine and apomorphine also give colors with Vitali's test that might be mistaken for those given by atropine. According to Pohl, physostigmine and pilocarpine also interfere with Vitali's atropine test. By extracting the alkaline aqueous solution with pure carbon disulphide, atropine which is alone soluble can be separated from the other two insoluble alkaloids.

[1] R. Eder: Reactions for the Detection of Atropine and Other Related Mydriatic Alkaloids. Schweizer Apotheker-Zeitung 1916.

[2] Vitali: L'Orosi 1880 No. 8 and Archiv d. Pharmaz. (3) 18 (1881) No. 37, 307.

[3] Eumydrine, formed by treating atropine methyl bromide with silver nitrate, is atropine methyl nitrate, white crystals soluble in water and alcohol and melting at 163°:

$C_{17}H_{23}NO_3.CH_3Br + AgNO_3 = AgBr + C_{17}H_{23}NO_3.CH_3NO_3$ (Eumydrine).

2. **Gulielmo's[1] Odor Test.**—Heat a little atropine in a small, dry test-tube over a low flame until a white vapor appears. At the same time an agreeable odor, recalling the perfume of black-thorn blossoms, can be detected. Then add about 1 cc. of concentrated sulphuric acid and heat until the acid begins to darken. Without cooling, dilute at once with 2 cc. of water added from a small graduate. During foaming this odor will be still stronger. A positive test is given by 0.01 gram of atropine. To test the residue from the ether extract of the aqueous alkaline solution, dissolve it in a littler ether, evaporate this solution in a test-tube put in a warm water-bath, and examine the residue. Mandelic acid derivatives, homatropine (see page 182) and euphthalmine, give the odor of benzaldehyde in this test, whereas the flower perfume of the tropic acid derivatives is said to be due to the cleavage-product atropic acid (Gadamer), or styrole (Kondakow), or phenyl-acetaldehyde (Rosenthaler).

3. **Color Reactions.**—Atropine differs from most of the alkaloids in not giving, at least in the cold, any striking colors that are characteristic with reagents usually employed for this purpose, such as concentrated sulphuric and nitric acid, and the reagents of Froehde, Erdmann, Marquis and Mecke. According to Wasicky,[2] perhydrol-sulphuric acid and p-dimethyl-amino-benzaldehyde-sulphuric acid (dimethyl-amino-benzaldehyde = $(CH_3)_2N.C_6H_4.CHO$) are exceptions.

(a) **Perhydrol-Sulphuric Acid (Schaer's[1] Reagent) Test.**—Atropine, hyoscyamine, homatropine, scopolamine and cocaine give a fine color-reaction with this reagent. If a few drops of perhydrol-sulphuric acid are added to a particle of the alkaloid, after 0.5 minute an intense leaf-green color beginning at the margin appears, becoming olive-green after a few minutes and finally discolored or brown-green.

(b) **Para-dimethyl-amino-benzaldehyde-Sulphuric Acid Test.**[3]—Add a drop of the reagent to a small quantity of atropine, hyoscyamine or scopolamine and warm gently, best upon an asbestos-plate. A red color appears, becoming in time an intense cherry-red to violet and remaining unchanged for a day. According to Wasicky, this is as delicate as the physiological test. Homatropine, cocaine and tropacocaine do not give this reaction.

[1] Zeitschr. f. analyt. Chemie 1863, 404.

[2] R. Wasicky: A New and very Delicate Color Reaction for Atropine, Hyoscyamine and Scopolamine. Zeitschr. f. analyt. Chemie 54 (1915), 393.

[3] See under "Preparation of Reagents" page 641.

Morphine and codeine even in the cold give at once a bright red color but it lacks the violet tone. Atropine gives this color in the cold only after a few hours. Quinine gives a red-brown color, physostigmine leaf-green, narcotine and papaverine orange, and veratrine deep green upon gentle warming.

4. Alkaline Reaction Test.—In aqueous solution, atropine, hyoscyamine and homatropine turn red litmus paper blue and also react with phenol-phthalein. Place a trace of alkaloid upon a strip of phenol-phthalein paper, add a drop of absolute alcohol, and allow it to evaporate. No color will appear. Then add a drop of water. The paper will at once turn red.

Cocaine, morphine, codeine and pilocarpine turn red litmus paper blue but have no action upon phenol-phthalein paper. Obviously, alkalies or alkaline carbonates should not be present. In case the ether residue contains the latter, it should be redissolved in the purest chloroform, the solution passed through a dry paper and then evaporated. This residue may then be tested with phenol-phthalein paper.

5. Gerrard's[1] Test.—Add about 2 cc. of a 1 per cent. solution of mercuric chloride in 50 per cent. alcohol to 1 milligram of atropine. Warm very gently and a yellow precipitate of mercuric oxide, becoming red upon boiling, will appear:

$$2C_{17}H_{23}NO_3 + H_2O + HgCl_2 = 2C_{17}H_{23}NO_3.HCl + HgO.$$

Hyoscyamine and homatropine also give Gerrard's test. This reaction fails with too great dilution.

In this test brucine, strychnine, veratrine, aconitine, coniine, caffeine and quinine give white precipitates. Those produced by morphine, and codeine are pale yellow.

6. Eder's[2] Micro-Tests with Bromine Water and Bromo-Potassium Bromide.[3]—Place a drop of a solution of the alkaloid in 0.5 n-sulphuric acid upon a glass slide and then allow a drop of the reagent to fall into this solution. The mixture should not be stirred. Several minutes after precipitation, put on a cover-glass without disturbing crystal formation. A microscope magnifying 180–250 times should be used in studying the forms of crystals. In the test given by atropine with saturated bromine water, the rather large prisms are especially characteristic. These are usually pale yellow, flat rods very regularly formed with the ends frequently cut off

[1] A. W. Gerrard: Pharm. Journ. and Trans. (3) 14 (1884), 718.

[2] R. Eder: Loc. cit. page 471.

[3] See "Preparation of Reagents" page 641.

obliquely. The test with bromo-potassium bromide is carried out in the same manner as that with bromine water and is more delicate than the latter. With a magnification of 200 times, small, pale yellow needles, spindle to cylindrical in form, also frequently in star or Y-shaped clusters, may be seen. Bromine water and bromo-potassium bromide also produce characteristically shaped crystalline precipitates in hyoscyamine and homatropine solutions. For purposes of comparison, tests should always be made with pure alkaloids at the same time. Scopolamine gives yellow precipitates with both bromine reagents. Under the microscope they are seen to consist of yellow amorphous droplets. Only as exceptions do centers of crystallization appear in spots.

Since atropine and hyoscyamine sublime without decomposition *in vacuo*, according to Eder micro-sublimates[1] may also be employed in testing for these two bases which are especially suited for this micro-chemical test. In experiments with the bromine reagents, bromine gradually evaporates and with it crystals disappear completely. Addition of another drop of bromine water or bromo-potassium bromide to the clear colorless solution will cause anew the characteristic precipitations.

In addition to the mydriatic bases mentioned, almost all the other alkaloids dissolved in 0.5 n-sulphuric acid give precipitates with saturated bromine water and bromo-potassium bromide. With the single exception of the caffeine precipitate, all the other precipitates of these alkaloids are amorphous. Macroscopically they are either milky or flocculent in appearance, or they clump together in the manner of resins. Under the microscope, however, they do not consist of doubly refracting droplets.

7. Physiological Test.—The action of atropine in causing enlargement of the pupil can be employed for the recognition of this alkaloid, for this enlargement of the pupil is distinct in the human or the cat's eye with one drop of an atropine solution 1:130,000. To make this test, dissolve the residue from the ether extract of the aqueous alkaline solution in 4–5 drops of extremely dilute sulphuric acid and introduce a drop of this solution into the conjunctival sac of one eye of a cat or dog, comparing the width of the two pupils. The eye of the rabbit is not suitable for this test.

Individual substances exerting mydriatic action upon the eye are very different with regard to speed of reaction, duration and intensity. Atropine, for example, is stronger and much more persistent in its action than homatropine. Cocaine enlarges the pupil but in much more concentrated solution than atropine and hyoscyamine and at the same time causes anaesthesia. The action of l-hyoscy-

[1] See "Micro-sublimation" in Chapter V, page 471 of this book.

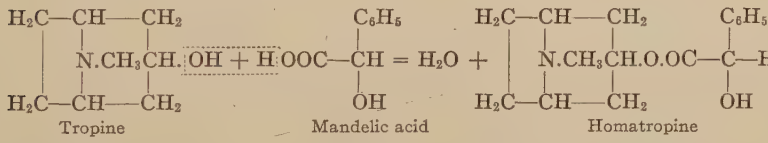
amine is nearly twice as strong as that of atropine and 12–18 times stronger than that of d-hyoscyamine. According to Joachimoglu,[1] scopolamine in the eye of the cat is about 10 times more active than atropine. Even 0.0002 mg. of atropine is said to produce mydriatic action in the human or cat's eye (Feddersen).

Notes.—Ptomaines exhibiting pronounced mydriatic action have repeatedly been found in putrefied flesh of fish. These extraordinarily toxic ptom-atropines are regarded by different investigators as the cause of poisoning from meat and especially from fish.

8. Gold Chloride Test.—The alkaloids possessing mydriatic properties may be distinguished from one another by preparing and characterizing their gold chloride double salts which crystallize well. This subject is more fully treated among the reactions of scopolamine (see page 185).

HOMATROPINE

Homatropine, $C_{15}H_{21}NO_3$, is the tropyl ester of phenyl-glycolic or mandelic acid. The hydrochloride of this base is obtained by heating tropine mandelate for several days in the water-bath with dilute hydrochloric acid, the latter acting as a dehydrating agent. The product of this reaction is then made alkaline with potassium carbonate and the free homatropine base precipitated as an oil is extracted with chloroform:



Distillation of the solvent leaves homatropine as an oil that crystallizes with difficulty. The free base forms colorless, hygroscopic prisms melting at 93.5–98.5°. On the other hand, homatropine hydrobromide, $C_{15}H_{21}NO_3.HBr$, employed in medicine, crystallizes very easily from water, forming wart-like clusters that dissolve readily in water and with difficulty in alcohol.

Detection.—Pure homatropine differs from atropine and hyoscyamine in not giving Vitali's test. The residue obtained by evaporating upon the water-bath even 0.01 gram of homatropine hydrobromide with fuming nitric acid has only a faint yellowish

[1] Joachimoglu: Berliner klinische Wochenschr. 52 (1915), 911.

color.[1] When cooled and treated with alcoholic potassium hydroxide solution, this residue acquires a yellow-red to red-yellow color.

If the odor test is made in the manner described for atropine, that is, without an oxidizing agent, homatropine gives an odor like that of benzaldehyde. Therefore it is easy to distinguish tropic acid derivatives by an odor like that of a flower or honey from mandelic acid derivatives, such as homatropine, by the benzaldehyde odor.

Homatropine is a strong base and consequently like atropine and hyoscyamine gives Gerrard's test. It also gives the test with phenolphthalein paper. Although giving the test with perhydrol-sulphuric acid, according to Wasicky homatropine does not respond to the test with p-dimethyl-amino-benzaldehyde and sulphuric acid. This serves to differentiate it from atropine, hyoscyamine and scopolamine.

With regard to its physiological properties, the action of homatropine upon the pupil of the eye is about as strong as that of atropine. The effect, however, disappears much more quickly, that is, in 12–24 hours, whereas the action of atropine may persist for 8 days. Consequently when it is a matter of producing enlargement of the pupil and having the action soon over, homatropine is to be preferred to atropine, because it affects accommodation slightly and only for a short time.

SCOPOLAMINE

Laevo-scopolamine, $C_{17}H_{21}NO_4.H_2O$, remains in the mother-liquors in the preparation of hyoscyamine and atropine from henbane seeds and those of Datura Stramonium. Larger quantities of scopolamine occur in the leaves of certain species of Duboisia and smaller quantities together with inactive scopolamine in the root of Scopolia japonica, Scopolia atropoides and other species of Scopolia. The root of Atropa Belladonna is said to contain very small quantities of scopolamine. (E. Schmidt.)

Properties.—Laevo-scopolamine crystallizes only with difficulty. It is deposited from a chloroform solution, when the solvent is distilled, as a viscous syrup. With some difficulty by means of ether this can finally be made to give colorless crystals melting at 59°. If crystallized scopolamine is kept over sulphuric acid, it changes to an amorphous, colorless, transparent mass. It dissolves rather easily in water, at least more easily than atropine. In alcohol, ether and chloroform it is freely soluble. The alcoholic solution of natural

[1] According to R. Eder (loc. cit.) the residue is colored distinctly yellow, if somewhat larger quantities of pure homatropine are present.

scopolamine is laevo-rotatory. If a little sodium hydroxide solution is added to this alcoholic solution, scopolamine gradually changes into optically inactive scopolamine. The latter probably bears the same relation to the natural laevo-rotatory base that inactive atropine does to active hyoscyamine.

Constitution of Scopolamine and Scopoline

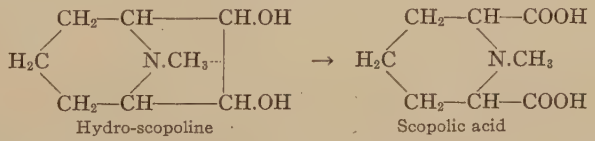
A warm solution of barium hydroxide brings about the hydrolytic cleavage of scopolamine into the base scopoline and the nitrogen-free constituent tropic acid:

$$\underset{\text{Scopolamine}}{C_{17}H_{21}NO_4} + H_2O = \underset{\text{Scopoline}}{C_8H_{13}NO_2} + \underset{\text{Tropic acid.}}{C_9H_{10}O_3}$$

When boiled for a longer time with considerable barium hydroxide solution, scopolamine undergoes cleavage into scopoline and atropic acid. Tropic acid, the first product of hydrolytic cleavage, loses a molecule of water and passes into atropic acid (E. Schmidt):

$$\underset{\text{Scopolamine}}{C_{17}H_{21}NO_4} = \underset{\text{Scopoline}}{C_8H_{13}NO_2} + \underset{\text{Atropic acid.}}{C_9H_8O_2}$$

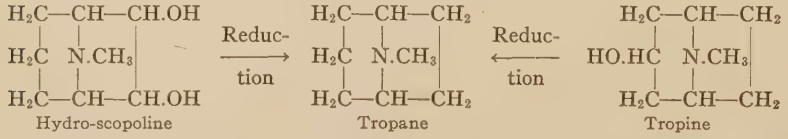
The constitution of tropic and atropic acid has been known for a long time. For some time it has been known, especially as a result of the researches of Schmidt, that one of the two oxygen atoms of scopoline belongs to a hydroxyl-group, whereas the other is in an ether-like union. Moreover a methyl-group is attached to nitrogen, since nor-scopoline is formed as the result of the cleavage of a methyl-group from scopoline through oxidation with permanganate, chromic acid, as well as through the action of bromine. By methylation of nor-scopoline it is possible to obtain scopoline again. Consequently the formula of scopoline may be resolved into $C_7H_9(NCH_3)(OH)(O<)$. Within recent years the researches of Hess,[1] carried out with great thoroughness, have explained the constitution of scopoline and scopolamine. By warming scopoline with a solution of hydrobromic acid in glacial acetic acid, Hess obtained an excellent yield of an addition-product of both substances, hydro-scopoline bromide, from which may be obtained by means of nascent hydrogen, that is, with zinc dust and sulphuric acid, at room-temperature or with zinc dust and glacial acetic acid at about 110°, dihydro-scopoline, $C_8H_{15}NO_2$. The latter, briefly called hydro-scopoline by Hess, can be oxidized by chromic-sulphuric acid upon the water-bath to the dibasic scopolic acid, an isomer of tropic acid but entirely different from it in properties. Scopolic acid is identical with N-methyl-piperidine-α,-α'-dicarboxylic acid obtained synthetically by Hess. This reaction can be understood on the basis of the following formula of hydro-scopoline:



Hydro-scopoline → Scopolic acid

[1] K. Hess: Degradation of Scopoline I. (with A. Suchier). Scopoline → Hydroscopoline → Scopolic Acid. Ber. d. Deutsch. chem. Ges. 48 (1915), 2057. II. Communication, Ibid. 49 (1916), 2337, 2745. III. Communication, Ibid. 51 (1918), 1007. IV. Communication, Ibid. 52 (1919), 1947.

As a result of these experiments, Hess has furnished clear proof that scopoline, and consequently scopolamine, are alkaloids of the piperidine group and that the C-atoms in question are attached on both sides to the nitrogen atom in the α-position. The conversion of hydro-scopoline into an oxygen-free base may be accomplished by heating it with hydriodic acid and considerable phosphonium iodide for several hours at 200°. In this manner the oxygen-free derivative of tropine, tropane, is obtained in about 25 per cent. yield:

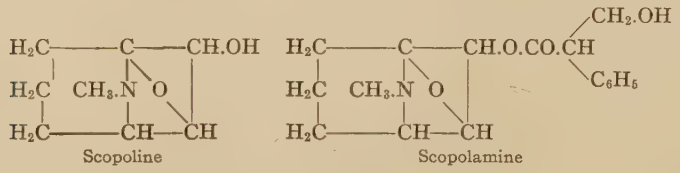


Hydro-scopoline — Tropane — Tropine

Therefore hydro-scopoline is dioxy-tropane.

The constitution of scopoline is explained by these experimental results and its membership in the tropic group of alkaloids is clearly established.

Upon the basis of results obtained by the degradation of scopoline according to Hofmann's method, Hess has proposed the following structural formulae for scopoline and scopolamine:



Scopoline — Scopolamine

Detection of Scopolamine

The mydriatic action of scopolamine is stronger than that of atropine and hyoscyamine. According to Joachimoglu,[1] scopolamine in the cat's eye is about 10 times more active than atropine. Scopolamine gives Gulielmo's odor test. As in the case of all tropic acid derivatives, when heated in the manner described for atropine, it gives a characteristic odor of black-thorn blossoms and honey. Since scopolamine is a weaker base than atropine and homatropine, it does not give Gerrard's test with mercuric chloride, nor does it color phenol-phthalein paper (see Atropine, page 179). On the other hand, it gives both of the tests recommended by Wasicky for mydriatic alkaloids. Toward the two bromine reagents of Eder, scopolamine differs from the other mydriatics in producing precipitates, consisting only of amorphous droplets that crystallize but seldom and irregularly. Therefore a test should always be made with pure scopolamine as a check. If the available material is

[1] Berliner klinische Wochenschr. 52 (1915), 911.

sufficient, the several mydriatic bases may be differentiated by preparing and identifying their gold chloride double salts, for these crystallize well, are difficultly soluble in cold water and have also a fairly sharp melting-point. To prepare these compounds, add a slight excess of chlor-auric acid solution to the alkaloid in question, for example, the residue from the ether solution, dissolved in dilute hydrochloric acid, and bring the precipitate into solution by warming. As the solution cools, the particular chloraurate will crystallize. The three most important compounds to be considered are:

Atropine Chlor-aurate.—This salt has the formula $C_{17}H_{23}NO_3.HCl.AuCl_3$. At first it is oily but gradually solidifies. The crystals are dull and melt at 135–137°.

Hyoscyamine Chlor-aurate.—This salt has the formula $C_{17}H_{23}NO_3.HCl.AuCl_3$. It crystallizes in gold-yellow leaflets that are highly lustrous and melt at 160–162°.

Scopolamine Chlor-aurate.—This salt has the formula $C_{17}H_{21}NO_4.HCl.AuCl_3$. It crystallizes from hot water in broad yellow prisms that are difficulty soluble, well-formed and lustrous. They melt at 210–214°. Frequently these crystals have more of a feathery appearance under the microscope.

Combination of Morphine with Scopolamine.—This combination, frequently employed at the present time by surgeons, greatly increases the narcotic effect of anaesthetics taken by inhalation, making it possible to conduct and maintain narcosis with a considerably lower concentration of anaesthetic in the air inhaled. This combination alone makes it possible to bring about a condition in which there is freedom from pain and consciousness is dulled so that even major operations can be carried on painlessly. From many quarters the "Morphine-Scopolamine-Narcosis" according to Schneiderlin-Korff, has been recommended even as a substitute for anaesthesia by inhalation.

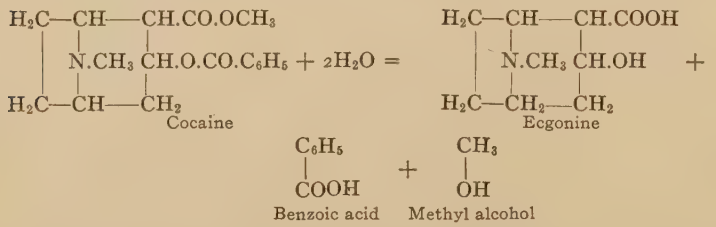
COCAINE

Cocaine, $C_{17}H_{21}NO_4$, occurs to the extent of 0.2–0.8 per cent. in coca leaves, the leaves of the South American shrub, Erythroxylon Coca. Other coca species, such as those of Java and the East Indies, also probably contain cocaine. Among the accompanying alkaloids, occurring in coca leaves from different sources, may be mentioned: cinnamyl-cocaine, $C_{19}H_{23}NO_4$; benzoyl-ecgonine, $C_{16}H_{19}NO_4.4H_2O$; cinnamyl-ecgonine, $C_{18}H_{21}NO_4$; tropa-cocaine, or benzoyl-pseudotropeine, $C_{15}H_{19}NO_2$; truxilline, $C_{19}H_{23}NO_4$; and hygrine, $C_8H_{15}NO$.

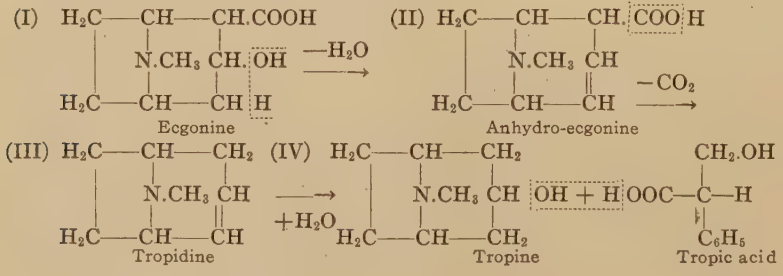
Properties.—Cocaine crystallizes best from hot acohol or petroleum ether and is obtained in large, colorless, monoclinic prisms melting at 98°. Its solutions

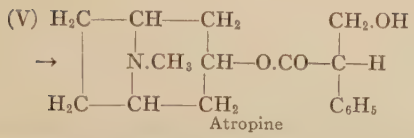
are laevo-rotatory and have a strong alkaline reaction. It has a faintly bitter taste producing upon the tongue temporary anaesthesia. Cocaine is difficultly soluble in water (1 : 700) but dissolves easily in alcohol, ether, benzene, chloroform and acetic ether. Dilute acids dissolve cocaine and usually form well-crystallized salts. The fixed alkalies, ammonia and alkaline carbonates precipitate the free base from solutions of its salts.

Constitution.—Cocaine is a monacid base, since it forms salts with one equivalent of acid. The most important cocaine salt is the hydrochloride, used in medicine and having the composition, $C_{17}H_{21}NO_4.HCl$. The tertiary nature of this alkaloid is shown by the fact that at 100° it combines with methyl iodide, forming the well-crystallized cocaine iodo-methylate, $C_{17}H_{21}NO_4.CH_3I$. It contains a methyl-group attached to nitrogen, that is, the group $N.CH_3$, because methylamine is split off when it is distilled with barium hydroxide. Moreover cocaine is the methyl ester of an acid and at the same time the benzoic acid ester of an alcohol, for when boiled with water it is decomposed into methyl alcohol and benzoyl-ecgonine. If mineral acids, barium hydroxide or alkalies are substituted for water, hydrolytic cleavage is carried farther with decomposition of benzoyl-ecgonine. In this way ecgonine, benzoic acid and methyl alcohol are obtained. The following equation expresses this cleavage on the basis of the structural formula of cocaine as proved by Willstätter:



Ecgonine (I), the constitution of which has been clearly determined, loses water when heated with phosphorus oxychloride giving anhydro-ecgonine (II). The latter loses carbon dioxide, when heated at 280° with fuming hydrochloric acid, and yields tropidine (III). If this compound is boiled with sodium hydroxide solution, it adds a molecule of water and is converted into the basic cleavage-product of atropine, that is, tropine (IV). Under the influence of dilute hydrochloric acid, tropine and tropic acid condense with formation of atropine (V). Consequently it is possible by synthetic methods to convert the alkaloid cocaine into the solanaceous alkaloid atropine:





Atropine

Physiological Properties and Toxic Action.—Cocaine is rapidly absorbed from all mucous surfaces. It has a two-fold action, that is, central and peripheral.

Even in small doses it exerts a central action, causing stimulation and increasing the efficiency of the brain and spinal cord (Kobert). Upon this property depends its use as a tonic and in producing exhilaration, as in the case of chewing coca leaves. After larger doses, this stimulation gives way to excitement and convulsions, followed finally by paralysis. Peripheral action, also local and produced by painting a solution of the alkaloid upon mucous surfaces and injecting it into other parts of the body, consists in deadening the sensitiveness of the sensory nerve endings.

Symptoms of cocaine poisoning that appear are: dryness of the throat and nose, difficulty in swallowing, nausea, emesis, burning pain in the stomach, attacks of colic or gastric spasms, and loss of appetite. Additional symptoms that may be mentioned are loss of smell and taste, impairment of the sense of hearing and smell, enlargement and rigidity of the pupils, and lowering of the power of accommodation. Larger doses may give rise to high spirits, a condition akin to drunkenness, hallucinations, followed by depression with melancholia, a state of anxiety, and ideas of persecution. There is quickening of the pulse, derangement of the heart, irregular respiration, and even paralysis of respiration. Kunkel considers 1.2 gram as the smallest lethal dose of cocaine.

Elimination.—According to experiments made by Wiechowski,[1] dogs eliminate by way of the kidneys 10–12 per cent. of cocaine unchanged, whereas rabbits do not eliminate this alkaloid in this manner. Similar experiments with atropine have shown that dogs eliminate 17–57 per cent. of this alkaloid in the urine. The characteristic decomposition products of these two alkaloids (ecgonine from cocaine and tropine from atropine) could not be found in the urine of the experimental animals. The behavior of these two alkaloids in the animal body is analogous in that both undergo profound decomposition. After rather large doses, undecomposed cocaine is said to appear in human blood, organs and urine. According to Proelss,[2] cocaine can be detected in cadaveric material after 14 days at the maximum; also ecgonine is said to be rapidly separated in the living organism. Further details with regard to these matters are given below.

Detection of Cocaine

Ether, chloroform or benzene will extract cocaine from an alkaline aqueous solution. Provided the examination is confined to cocaine,

[1] W. Wiechowski: Fate of Cocaine and Atropine in the Animal Body. Arch. f. exper. Pathol. u. Pharmakol. 46 (1901), 155.

[2] H. Proelss: Contributions to the Detection of Alkaloids, Glucosides and Bitter Principles in Forensic-Chemical Investigations. Apotheker-Zeitung 16 (1901), 288, 306, 316, 325.

and there is no need of considering morphine, render the aqueous tartaric acid solution alkaline not with sodium hydroxide solution but with sodium carbonate, or better sodium bicarbonate, for cocaine is an ester and is easily decomposed by caustic alkalies. Most of the alkaloidal reagents will precipitate cocaine even from very dilute solutions of its salts. The reagents especially sensitive are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, phospho-molybdic acid, phospho-tungstic acid, gold chloride, plantinic chloride and picric acid. Pure concentrated sulphuric and nitric acid, as well as Erdmann's Froehde's and Mandelin's reagent, dissolve cocaine without color. This behavior differentiates cocaine from various other alkaloids. Cocaine picrate melts at 165–166°.

Special Reactions

1. Precipitation Test.—If 1–2 drops of potassium hydroxide solution are added to an aqueous solution of a cocaine salt not too dilute, it will become milky. First oil-drops and later fine crystalline needles of the free base separate:

$$C_{17}H_{21}NO_4.HCl^1 + KOH = C_{17}H_{21}NO_4 + H_2O + KCl.$$

In applying this test to the residue from the ether extract of the aqueous alkaline solution, dissolve it first in a few drops of dilute hydrochloric acid and add potassium hydroxide solution drop by drop in excess, cooling well by setting in ice. Special care must be taken to have the alkaloid pure enough so that, after filtration, washing and drying, its melting-point (98°) can be determined.

Obviously, precipitation of cocaine from solutions of its salts by potassium hydroxide solution is not characteristic, for under these conditions most alkaloids are similarly precipitated.

2. Potassium Permanganate Test.—Add saturated potassium permanganate solution drop by drop to a concentrated aqueous solution of a cocaine salt. A violet, crystalline precipitate of cocaine permanganate will appear. In applying this test to the residue from the ether solution of II B, dissolve it first in a few drops of dilute hydrochloric acid, evaporate the solution upon the water-bath, and

[1] Cocaine hydrochloride crystallizes from a concentrated aqueous solution in fine prisms containing 2 molecules of water of crystallization which it easily loses. This salt crystallized from alcohol is anhydrous and is the officinal Cocainum Hydrochloricum having the composition $C_{17}H_{21}NO_4.HCl$.

test the solution of the dry residue in water with potassium permanganate solution (1:100).

3. **Chromic Acid Test.**—Add 5 per cent. chromic acid solution, or potassium dichromate solution of corresponding concentration, to a solution of a cocaine salt not too dilute. Each drop will produce a precipitate that will immediately disappear upon shaking the solution. Then add to the clear solution about 1 cc. of concentrated hydrochloric acid. This will produce a more or less crystalline, orange precipitate.

Only when the quantity of cocaine for examination is not too small, do tests 1–3 give a positive result admitting of no doubt. They are applicable therefore for the identification of cocaine in pharmaceutical examinations rather than for the purposes of forensic chemistry.

4. **Detection of Benzoyl-group.**—This test requires at least 0.2 gram of cocaine. Warm the cocaine for a few minutes in a small test-tube with 2 cc. of concentrated sulphuric acid in a boiling water-bath. Cool and dilute with water, adding it drop by drop and all the while keeping the mixture cold. A white crystalline precipitate of benzoic acid will appear. Filter, wash with a little ice-water and dry this precipitate. Benzoic acid may be identified by sublimation, or by determining its melting-point (120°), provided the quantity is sufficient. Benzoic acid may also be extracted with ether. Evaporate the ether solution and warm the residue with 1 cc. of absolute alcohol and the same quantity of concentrated sulphuric acid. The characteristic odor of ethyl benzoate will be detected:

$$C_6H_5.COOH + HO.C_2H_5 + H_2SO_4 = C_6H_5.COOC_2H_5 + (H_2SO_4.H_2O).$$

In doubtful cases make a similar test with pure benzoic acid as a check.

5. **Iodic Acid Test.**—Add a few drops of concentrated sulphuric acid to a trace of cocaine and then a small particle of potassium iodate free from iodide, or iodic acid. No color appears in the cold but, if this mixture is heated in a porcelain dish over a small flame until vapors of sulphuric acid come off, and just a little longer, there appear in succession and together brown, olive-green, blue and violet shades of color which issue from the particle of iodate and immediately disappear. Finally vapors of iodine are also given off. According to Gadamer, this play of colors is really due to the presence of the benzoyl-group in the cocaine molecule, for pure benzoic acid gives it especially clearly. By this iodic acid test it is said to be possible to detect even 0.05 mg. of cocaine. But other alkaloids also give

intense colorations with sulphuric acid and potassium iodate and usually even in the cold. Under the same conditions ecgonine gives cherry to purple-red colors that finally become dirty violet.

6. Physiological Test.—Dissolve the given material (the residue from the ether extract of the aqueous alkaline solution) in a few drops of dilute hydrochloric acid and evaporate the solution to dryness upon the water-bath. Dissolve the residue in a little pure water and place a drop of this solution upon the tongue. If cocaine is present, it will produce temporary anaesthesia of the tongue.

R. Kobert ("Intoxikationen") has found rather small frogs sufficiently sensitive for the physiological identification of cocaine. The effects to be observed are dilatation and fixedness of the pupil, enlargement of the palpebral fissure and also stimulation of the nervous system. Administer about the same quantity of cocaine hydrochloride to another animal as a check.

The fact that cocaine does not give Vitali's test differentiates it from the alkaloids atropine, hyoscyamine and scopolamine which are also mydriatic in action. Moreover, since cocaine is easily soluble in petroleum ether, whereas atropine is not, this solvent may be used in extracting the former alkaloid.

Detection of Cocaine and Novocaine in the Cadaver

According to different statements in the literature, it is said that cocaine may be detected in the cadaver even 14 days after death. As the result of an examination for the purpose of determining whether a solution of cocaine instead of novocaine had been injected into two female patients, who had died with symptoms of cocaine poisoning, Popp[1] was unable to establish the presence of either cocaine or novocaine in the organs received 5 days after death. In connection with this investigation Popp together with Mannich and Ellinger conducted experiments upon rabbits, injecting into them varying quantities of cocaine and novocaine. The animals were killed at different intervals after injection and the extracts from their organs tested for cocaine by their anaesthetizing action and by the potassium permanganate and iodic acid test. Only in a single instance was cocaine detected at the place of injection 3 hours after death. Novocaine in doses of 100 mg. for each animal was also detected only at the place of injection but even when putrefaction was beginning. Separation of cocaine and novocaine, carried out according to the method of Kircher-Hoechst (see below), succeeded only with quantities of at least 2 mg. All the older statements with regard to the detection of cocaine in the cadaver after longer periods should be viewed with scepticism. Cocaine probably belongs to those poisons which

[1] G. Popp: Detection of Cocaine and Novocaine in Cadaveric Material. Abstract of an address delivered at the principal meeting of the society of independent public chemists in the Chemiker-Zeitung 1922.

can very seldom be detected in the cadaver. Apparently the cleavage of the methoxyl and benzoyl-group from the cocaine molecule in the animal organism is accomplished with the greatest ease (Heiduschka).

In the detection of novocaine, use may be made of the primary, aromatic amino-group which can be diazotized and then coupled with formation of an azo-dyestuff. Its detection in urine is usually attempted in this manner.

Detection of Cocaine in Novocaine

(Kircher[1])

Acidify the given novocaine-cocaine solution with dilute hydrochloric acid, cool and add drop by drop 0.5 per cent. sodium nitrite solution until after standing 15 minutes it still turns starch-iodide paper blue. Now add to the diazotized solution enough filtered aluminol solution (aluminium salt of β-naphthol-disulphonic acid) rendered alkaline with sodium carbonate, transfer the deep red mixture to a separating funnel, and repeatedly extract with sufficient quantities of benzine. The latter takes up cocaine unchanged by diazotization together with small quantities of dyestuff. To remove the dyestuff, repeatedly wash the cocaine-benzine solution with small quantities of water and then extract with an excess of dilute hydrochloric acid. Evaporate the aqueous solution of cocaine hydrochloride thus obtained upon the water-bath to the point of crystallization and test these crystals as described on page 188 for cocaine.

PHYSOSTIGMINE

Physostigmine, or eserine, $C_{15}H_{21}N_3O_2$, occurs in the cotyledons of the seeds of Physostigma venenosum to the extent of about 0.1 per cent. together with eseridine, $C_{15}H_{23}N_3O_3$. The former has been detected also in pseudo-Calabar beans, Cali-nuts.

Properties.—Physostigmine is obtained in large rhombic crystals, melting at 105°, by spontaneous evaporation of its benzene solution. Though only slightly soluble in cold water, it dissolves freely in alcohol, ether, benzene and chloroform. Physostigmine solutions are strongly alkaline, nearly tasteless and laevo-rotatory. If physostigmine is heated for some time at 100°, it turns red and then gives red solutions with acids. This alkaloid, notwithstanding the presence of three nitrogen atoms, is only a monacid base forming salts with one equivalent of acid that easily undergo decomposition and crystallize with difficulty. Physostigmine sulphate, $(C_{15}H_{21}N_3O_2)_2.H_2SO_4$, and physostigmine salicylate, $C_{15}H_{21}N_3O_2.C_6H_4(OH)COOH$, the salts of this alkaloid that are most stable and most easily obtained in crystalline condition, are used as myotics in ophthalmology and also as purgatives. Caustic alkalies, sodium carbonate and bicarbonate set physostigmine free from its salts, without causing a precipitate when the solution is quite dilute, so that the free base can be extracted by ether or chloroform. Since

[1] Private information from Dr. Kircher of the laboratory for pharmaceutical investigation of the Color Works at Höchst on the Main, kindly submitted in a private communication of Dr. G. Popp of Frankfort on the Main.

physostigmine forms an iodo-methylate, $C_{15}H_{21}N_3O_2.CH_3I$, with methyl iodide, it is a tertiary base; and since it gives methyl amine together with other bases when distilled with potassium hydroxide, the group: $N.CH_3$ must be present. The latter gives physostigmine its basic properties. Moreover it contains a hydroxyl-group.

Physiological Properties.—Physostigmine exhibits central and peripheral action, the former consisting of stimulation of the brain and spinal cord with subsequent paralysis. The peripheral action of physostigmine is strikingly like that of pilocarpine and muscarine with respect to the eye, salivary glands, gastro-intestinal tract, etc. As a result of this action, contraction of the pupils, which may persist for 10–24 hours, occurs, glandular secretion is heightened, and the action of the heart and intestines is increased. Consequently its action is just the reverse of that of the mydriatic alkaloids. This explains how atropine in not too small doses can at least partially neutralize the effects of physostigmine. Internal administration of physostigmine does not always cause contraction of the pupils (myosis) either in man or horses. Some of the usual symptoms that may be mentioned are: watering of the eyes, flow of saliva, severe sweating, diarrhoea, weakness of the heart, slowing of the pulse, increase of blood-pressure, uneasiness, and sometimes maniacal attacks. Death ensues from paralysis of respiration.

Physostigmine is easily absorbed and rather quickly eliminated in the urine, bile, saliva and milk. Without doubt a large part of the alkaloid is destroyed in the body. Detection of physostigmine in the urine is frequently possible.

A noteworthy result is the effect of magnesium salts in counteracting trembling of the limbs (tremor) occasioned by physostigmine. On the contrary they are without influence upon contraction of the pupils Consequently magnesium salts are useful in certain cases as an antidote for eserine (Joseph[1]).

Detection of Physostigmine

Since physostigmine is easily decomposed by strong bases, the aqueous tartaric acid solution in the Stas-Otto process should first be covered with ether, then rendered alkaline with sodium hydroxide solution, and at once shaken vigorously. In this way physostigmine, if present, is dissolved by ether and removed from the destructive action of caustic alkali. Provided there is no reason to include morphine in the examination, sodium bicarbonate may be substituted for sodium hydroxide. When acid and alkaline solutions of physostigmine are warmed in contact with air, or even exposed to light, they turn red. This is due to formation of rubreserine. Because of the ease with which physostigmine undergoes decomposition, material such as vomitus, consisting of gastic as well as intestinal contents, liver, bile, blood and urine, should be acidified with tartaric acid and

[1] D. R. Joseph: Inhibiting Influence of Magnesium upon Certain Toxic Effects of Eserine. Amer. Journ. Physiol. 23 (1909), 215.

extracted with alcohol in the dark at room temperature. Alcohol in the filtered extract, protected from light, should be distilled *in vacuo*, the residue filtered, the filtrate rendered alkaline with sodium bicarbonate and extracted with ether. To purify the residue from this ether extract, dissolve it in cold water containing tartaric acid, filter the solution, render alkaline with sodium bicarbonate, and again extract with ether. The residue from this ether solution should be tested for physostigmine.

Special Reactions

1. Concentrated nitric acid dissolves physostigmine with yellow color. Concentrated sulphuric acid dissolves it, according to the degree of purity, colorless or with yellow color.

2. **Ammonia Test.**—Dissolve physostigmine in water with a trace of sulphuric acid, then add a few drops of ammonia, and evaporate to dryness upon the water-bath. First a red color appears but the residue is blue. Should it show a tint of red, evaporate it once more with ammonia. The blue residue will dissolve in alcohol with a blue color. If acetic acid or mineral acid is added, the solution by transmitted light will appear litmus-violet, but by reflected light red, exhibiting at the same time a fine eosine-red fluorescence. If again rendered alkaline with ammonia, chloroform will dissolve the dyestuff with a green-blue color (Gadamer).

Gaubert's[1] Procedure.—If an aqueous physostigmine solution is allowed to stand for a month until it has taken on a deep blue color, addition of phthalic anhydride will produce blood-red fluorescence. This new substance may be obtained in dark blue crystals that dissolve in alcohol and ether, giving dark blue solutions that do not fluoresce. On the other hand, the aqueous solution appears nearly colorless by transmitted light but by reflected light exhibits brilliant blood-red fluorescence. According to Gaubert, this substance is more strongly fluorescent than any substance at present known.

3. **Rubreserine and Eserine Blue Test.**—If an aqueous solution of a physostigmine salt is shaken for some time in contact with air with an excess of potassium or sodium hydroxide solution, cleavage of carbon dioxide and methyl amine takes place with formation of the red dyestuff, rubreserine. This compound separates as red needles that become greenish blue upon further oxidation owing to

[1] P. Gaubert: A New Highly Fluorescent Substance obtained from Physostigmine. Compt. rend. de l'Acad. des sciences 149 (1909), 852.

formation of eserine blue. If barium hydroxide solution is substituted for alkaline hydroxide, a white precipitate first appears soon becoming red when shaken. Sometimes this change occurs even in the cold but invariably with heat.

4. Physiological Test.—The marked action of physostigmine in causing contraction of the pupil is very characteristic. To make this test, dissolve the residue from the ether solution in water containing a trace of hydrochloric acid and introduce this solution into the conjunctival sac of a cat. Even 0.1 milligram of physostigmine will produce noticeable contraction.

PILOCARPINE

Pilocarpine, $C_{11}H_{16}N_2O_2$, together with iso-pilocarpine and probably also pilocarpidine, is a constituent of true jaborandum leaves, the leaves of Pilocarpus pennatifolius,[1] a member of the Rutaceae family, indigenous to Brazil. False jaborandum leaves, the leaves of Piper reticulatum and of other species of Piper and Pilocarpus, also appear to contain pilocarpine in varying quantities. Genuine hairy jaborandum leaves yield about 1 per cent. of pilocarpine.

Properties.—The free base pilocarpine is usually obtained as a semi-liquid, viscous, non-volatile mass having an alkaline reaction. It dissolves but slightly in water, is freely soluble in alcohol, ether and chloroform, but insoluble in benzene. Solutions of pilocarpine and its salts are dextro-rotatory. This alkaloid is a strong base neutralizing acids and forming salts that are usually crystalline. Caustic alkalies, added to concentrated solutions of pilocarpine salts, precipitate the free base which redissolves in excess of precipitant. Solutions of sodium hydroxide, or sodium ethylate, cause molecular rearrangement of pilocarpine. This reaction runs more smoothly, if pilocarpine hydrochloride is heated for half an hour at 200°. The product of this change is iso-pilocarpine, isomeric and very likely stereo-isomeric with pilocarpine. Both isomeric pilocarpines differ in melting-points, solubilities and particularly in specific rotation. Iso-pilocarpine is less dextro-rotatory than pilocarpine and crystallizes in deliquescent prisms easily soluble in water and alcohol. The salts of the two bases also show similar differences:

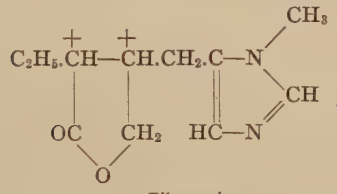
Pilocarpine nitrate: $C_{11}H_{16}N_2O_2.HNO_3$; mpt. 178°; $[\alpha]_D = +85.90°$.
Iso-pilocarpine nitrate: $C_{11}H_{16}N_2O_2.HNO_3$; mpt. 159°; $[\alpha]_D = +35.68°$.

Jowett[2] has succeeded in converting iso-pilocarpine into pilocarpine by the same reagent used in converting pilocarpine into iso-pilocarpine. Pure iso-

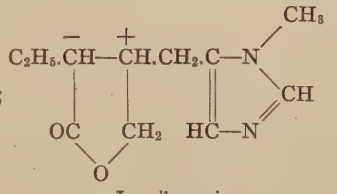
[1] According to the researches of H. A. D. Jowett, jaborine, described as another alkaloid in jaborandum leaves, is a mixture of iso-pilocarpine, pilocarpidine, a little pilocarpine and coloring-matter.

[2] H. A. D. Jowett: Constitution of Pilocarpine, Part I. Conversion of Iso-pilocarpine into Pilocarpine. Proc. Chem. Soc. 21 (1905), 172; Journ. Chem. Soc. London 87 (1905), 794.

pilocarpine, heated with alcoholic potassium hydroxide, gives a mixture of unaltered iso-pilocarpine and pilocarpine. The identity of the latter with pure pilocarpine was established by preparing the hydrochloride, melting at 201°, $[\alpha]_D = +92.8°$; and nitrate, melting at 177–178°. This reciprocal conversion of one alkaloid into the other strongly supports the idea of the stereo-isomerism of pilocarpine and iso-pilocarpine. Pilocarpine combines with one equivalent of acid, forming crystalline salts that are usually easily soluble in water and alcohol. Consequently pilocarpine is a monacid base. Pilocarpine hydrochloride, $C_{11}H_{16}N_2O_2.HCl$, usually in the form of laminated crystals, having a faint acid reaction and bitter taste, is used in medicine. It abstracts moisture from the air and therefore is deliquescent. Caustic alkalies and barium hydroxide solution cause temporary precipitation of the free base only from concentrated solutions of pilocarpine salts, for it redissolves in excess of precipitant forming salts of pilocarpic acid, $C_{11}H_{18}N_2O_3$. Mineral acids liberate from these salts pilocarpic acid which at once loses water and passes into its inner anhydride, pilocarpine. On the basis of this behavior pilocarpine is a lactone. By distillation with soda lime, Jowett obtained methyl-glyoxaline, dimethyl-glyoxaline and methyl-amyl-glyoxaline. According to this behavior, pilocarpine is a derivative of glyoxaline. In view of these facts, therefore, pilocarpine and iso-pilocarpine must be tertiary bases containing in the molecule a lactone and a glyoxaline ring. This confirms the structural formula first proposed by A. Pinner. Jowett explains the stereo-isomerism in the following manner:

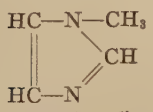


Pilocarpine

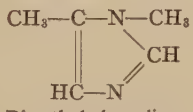


Iso-pilocarpine

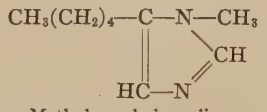
The glyoxaline bases obtained by means of soda lime are:



Methyl-glyoxaline



Dimethyl-glyoxaline



Methyl-amyl-glyoxaline

The unsaturated character of the glyoxaline ring probably accounts for the ease with which pilocarpine undergoes oxidation.

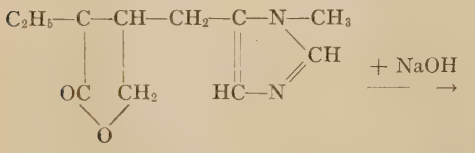
Physiological Action.—The effects of pilocarpine upon the animal organism very closely resemble those of nicotine except that in the case of pilocarpine peripheral effects preponderate. Pilocarpine in its action is antagonistic to atropine, for it increases the secretions, especially that of the saliva, sweat and of the intestinal mucosa. Secretions from all the other glands are increased, for example, from the lachrymal, bronchial and tracheal glands. Consequently pilocarpine is used in medicine to cause flow of saliva, perspiration, intestinal secretion and mucous secretion of the respiratory tract. The symptoms of pilocarpine poisoning mentioned by Kobert are: flow of saliva, sweating, weep-

ing, profuse secretion from the nose, emesis, diarrhoea, contraction of the pupils, indistinctness of vision, palpitation of the heart, retarded pulse, headache, dizziness, rattling in the trachea, and oedema of the lungs. There is copious formation of mucus in the lungs which may give rise to attacks of coughing and asphyxiation. Atropine is very useful as an antidote. Pilocarpine is easily absorbed and probably is quickly eliminated. In keeping with its chemical structure and on account of the ease with which it undergoes oxidation, it is probably decomposed in the human body, if not completely, at least in the main so that at most only traces of undecomposed pilocarpine can be detected in the urine and in the organs of the body.

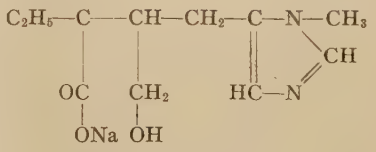
Detection of Pilocarpine

Ether, chloroform or benzene extracts pilocarpine from aqueous solutions that have been rendered alkaline with ammonia, or better with sodium bicarbonate. Evaporation of these solutions leaves a thick, non-crystalline, alkaline syrup or varnish. The general reagents especially sensitive to pilocarpine are: iodo-potassium iodide, phospho-molybdic acid, phospho-tungstic acid and potassium bismuthous iodide.

If the examination follows the usual procedure of the Stas-Otto process and the tartaric acid solution is rendered alkaline with sodium hydroxide solution, the lactone ring of pilocarpine, as soon as this base is set free, is broken and it passes into solution as sodium pilocarpate:



Pilocarpine



Sodium pilocarpate

If the solution is acidified, the reaction is reversed. Free pilocarpic acid loses water and forms its lactone pilocarpine which remains in solution as a salt. If this acid solution is made alkaline with ammonia or sodium bicarbonate, free pilocarpine may be extracted with ether or chloroform and the residue from this extract examined by Helch's test or biologically.

Special Reactions

1. **Helch's[1] Test.**—Put a particle of potassium dichromate and 1–2 cc. of chloroform into a test-tube. Then add pilocarpine itself, or its solution, and 1 cc. of 3 per cent. hydrogen peroxide. Shake for several minutes without interruption. The mixture yellowish at first gradually darkens and in 5 minutes is dark brown. Depending upon the amount of pilocarpine, the chloroform is blue-violet, dark blue or indigo-blue but the upper aqueous solution gradually fades. The chloroform is an intense blue from 0.01 gram of pilocarpine and blue-violet from 0.001 gram and less. Sometimes the color persists for a day. This reaction, however, is not confined exclusively to pilocarpine.

Notes.—Even without hydrogen peroxide, 0.01 gram of apomorphine colors chloroform blue-violet. Strychnine gives a barely perceptible bluish tint that fades entirely within a few minutes. Antipyrine gives a blue color in chloroform only after acidification of the hydrogen peroxide. Salipyrine and migrainine behave in the same manner.

2. **Physiological Test.**—Dissolve the residue from the ether or chloroform solution in water containing a drop of hydrochloric acid. Evaporate this solution upon the water-bath to remove excess of hydrochloric acid and introduce the pure aqueous solution of the residue into the conjunctival sac of a cat's eye. If pilocarpine is present, it will cause contraction of the pupil. The human eye is also very sensitive to the action of this alkaloid. Muscarine, nicotine and physostigmine, as well as pilocarpine, cause contraction of the pupil, or myosis.

Thus far fatal poisoning from this alkaloid has not occurred and nothing is known with regard to the possibility of its detection in the cadaver.

CODEINE

Codeine, $C_{18}H_{21}NO_3.H_2O$, occurs to the extent of 0.2–0.8 per cent. in almost all kinds of opium. It is the methyl ether of the monacid phenol morphine. Eucodine[2] is a codeine derivative.

[1] H. Helch: Reactions of Identification of Pilocarpine Hydrochloride. Pharm. Post. 35 (1902), 289; 39 (1906), 313.

[2] Codeine bromo-methylate, $C_{17}H_{18}O_2(OCH_3)N.CH_3Br$, obtained from anhydrous codeine and methyl bromide dissolved in chloroform, has been introduced into medicine under the name eucodine. It is said to be much less toxic than codeine, that is, convulsive action has been greatly diminished. Eucodine forms lustrous prisms having a bitter taste and also dissolving easily in cold water. Its solutions have a neutral reaction (distinction from codeine). It melts with decomposition at 261°.

Properties.—Codeine crystallizes from pure water, or from ether containing water, in colorless, transparent octahedrons that are often very large and contain 1 molecule of water. But from its solution in anhydrous ether or benzene it appears as smaller, rhombic crystals that are anhydrous, very lustrous and melt at 155°. The hydrated crystals lose crystal water at 100°. Codeine dissolves at 15° in 118 parts and at 100° in 15 parts of water, giving solutions that have a bitter taste, a strong alkaline reaction, and turn the plane of polarized light to the left. Codeine differs from most of the other alkaloids, especially from morphine, by its relatively high solubility in water. Hydrated codeine dissolves very readily in alcohol, ether, benzene and chloroform but very slightly in carbon disulphide. Codeine is as soluble in ammonia as in water but almost insoluble in sodium and potassium hydroxide solution. In this respect it differs from morphine which dissolves easily in solutions of caustic alkalies.

Preparation by Methylating Morphine.—1. Morphine is methylated when heated at about 60° in methyl alcohol solution with methyl iodide in presence of sodium or potassium hydroxide:

$$\underset{\text{Morphine}}{C_{17}H_{18}O_2N(OH)} + KOH \rightarrow \underset{\text{Potassium morphinate}}{C_{17}H_{18}O_2N(OK)} + CH_3I \rightarrow \underset{\text{Codeine}}{C_{17}H_{18}O_2N(OCH_3)}.$$

2. The same result may be accomplished by heating morphine, dissolved in a methyl alcohol solution of sodium or potassium hydroxide, with potassium or sodium methyl sulphate for several hours under a reflux:

$$C_{17}H_{18}O_2N(OK) + CH_3.O\,SO_2.OK = C_{17}H_{18}O_2N(OCH_3) + K_2SO_4.$$

3. Gently heat a solution of sodium morphinate in methyl alcohol with dimethyl sulphate. Sodium methyl sulphate will separate out but codeine will remain in solution:

$$C_{17}H_{18}O_2N(ONa) + (CH_3)_2SO_4 = C_{17}H_{18}O_2N(OCH_3) + CH_3O.SO_2.ONa.$$

4. Codeine is also formed as the result of the action of diazo-methane in the nascent condition upon potassium morphinate:

$$C_{17}H_{18}O_2N(OK) + CH_2N_2 + H_2O = N_2 + C_{17}H_{18}O_2N(OCH_3) + KOH.$$

In these reactions of synthesis, the hydrogen of the phenolic hydroxyl-group of morphine is replaced by methyl.

Constitution.—Codeine is a strong monacid base which completely neutralizes acids and usually forms crystalline salts. Codeine phosphate, $C_{17}H_{18}O_2(OCH_3)$-$N.H_3PO_4.H_2O$, separates upon addition of alcohol to a saturated solution of codeine in officinal phosphoric acid in short prisms, or as a white, crystalline powder. It has many uses in medicine. In water it dissolves with ease (1: 3.2) but is only slightly soluble in alcohol. Codeine is a tertiary base, since it combines with methyl or ethyl iodide, when heated at 100° in presence of a little alcohol, forming the finely crystallized codeine iodo-methylate, $C_{17}H_{18}O_2(OCH_3)$-$N.CH_3I$, or codeine iodo-ethylate, $C_{17}H_{18}O_2(OCH_3)N.C_2H_5I$, from which moist silver oxide sets free the unstable ammonium bases, codeine methyl hydroxide, $C_{17}H_{18}O_2(OCH_3)N.CH_3.OH$, and the corresponding ethyl derivative. When heated with potassium hydroxide, codeine gives off methyl amine, together with a little trimethylamine, proving that the codeine molecule contains the group $= N.CH_3$.

Physiological Action.—The particular difference between codeine and morphine is that the narcotic action of the former upon the brain-centers is very much reduced but continues to affect the respiratory center. Within recent years use of codeine as a cough remedy has increased considerably. As a cough remedy, a dose of 0.03–0.06 gram of codeine phosphate acts upon man as effectively as 0.005–0.01 gram of morphine. Its toxicity is about 20 times less than that of morphine. Even large doses do not produce true narcosis but the symptoms observed are: restlessness, slight muscular twitching, as well as mydriasis, except in certain cases of idiosyncrasy. Codeine is less adapted for alleviating pain and as a sedative (Hans Meyer and R. Gottlieb). Doses of 0.8 gram of codeine gave rise to: trembling, muscular weakness, visual disturbances, myosis, feeling of dizziness, quickening of the pulse, and collapse (Lewin). In addition, marked dilatation of the pupils, persisting several days, flow of saliva, and very defective respiration appear (Kobert). Following subcutaneous injection, codeine may be found in the stomach, intestines, blood and liver. Experiments by Bouma[1] upon dogs showed in one case that 77.1–89.6 per cent. of codeine, administered in daily injections of 0.2–0.4 gram of codeine phosphate over rather a long period, was eliminated unchanged in the urine and faeces together; and 81.1–83.1 per cent. in a second case. Although the body, as the result of continued administration of codeine, does not acquire the power of decomposing this alkaloid, it gives no indication of becoming habituated to it. On the contrary, the fact was established that the experimental animals exhibited increased sensitiveness. Probably there is no codeinism. Elimination of codeine as such proceeds by way of the urine and faeces, but principally by the former. After subcutaneous injection, codeine was found in the stomach, intestines, blood and liver.

Detection of Codeine

Codeine can be extracted from the aqueous alkaline solution by ether, benzene and chloroform. This alkaloid appears in the ether residue usually as a non-crystalline varnish in which it may be detected by the following tests:

1. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves pure codeine without color. After several days standing in the cold, or at once after gentle heating, the solution becomes reddish to brownish violet. If the solution of codeine in concentrated sulphuric acid is heated upon a watch-glass to about 150°, that is, until white vapors appear, and cooled, it will give a deep red color with a drop of concentrated nitric acid. This test like that of Pellagri (3) is due to formation of apomorphine by heating the sulphuric acid solution of codeine and detection of this base with nitric acid:

$$\underset{\text{Codeine}}{C_{17}H_{18}O_2(OCH_3)N} + H_2SO_4 = \underset{\text{Apomorphine}}{C_{17}H_{17}O_2N} + CH_3.O.SO_2.OH + H_2O.$$

[1] J. Bouma: Experiments upon Habituation to Codeine. Arch. f. exper. Path. u. Pharm. 50 (1903), 353.

2. Nitric Acid Test.—Concentrated nitric acid dissolves codeine with yellow color.

3. Pellagri's Test.—Both codeine and morphine give this test. Dissolve codeine in 3–4 cc. of concentrated hydrochloric acid and 3–4 drops of concentrated sulphuric acid. Evaporate this solution in a porcelain dish upon the water-bath, until the odor of hydrochloric acid is gone, and then allow it to stand about half an hour longer upon the boiling water-bath. Dissolve the dirty red or violet residue in about 3 cc. of water, add a few drops of dilute hydrochloric acid and solid sodium bicarbonate to a faint alkaline reaction. Then add drop by drop, at first only 2 drops, dilute alcoholic iodine solution and shake well for several minutes after each addition. Presence of codeine is indicated by an emerald-green color that frequently is intensified by another drop of iodine solution. Shake in a test-tube with 2–3 cc. of ether. The solvent will have a fine red color, whereas the aqueous solution will remain green. The following equation explains this test:

$$C_{17}H_{18}O_2(OCH_3)N + HCl = C_{17}H_{17}O_2N + CH_3.Cl + H_2O.$$

4. Oxidation Test.—Mix a little codeine upon a watch-glass with 4 times the quantity of finely powdered potassium arsenate (KH_2AsO_4). Add a few drops of concentrated sulphuric acid and then warm this mixture very gently over a small flame. The acid will have a deep blue color or, if the codeine is not quite pure, more of a bluish to reddish violet. If water or sodium hydroxide solution is added, the blue color will change to orange-yellow. Excess of potassium arsenate does not affect the test.

A trace of ferric chloride solution may be substituted for potassium arsenate as an oxidizing agent. Sulphuric acid containing 1 drop of ferric chloride solution to 10 cc. of acid is prescribed by the German Pharmacopoeia to detect codeine in codeine phosphate.

5. Froehde's Test.—This reagent dissolves codeine with a yellowish color which soon changes to green and finally to deep blue. Gentle warming of the solution over a very small flame will hasten this change of color.

R. Mauch warms 2–3 drops of a chloral hydrate solution of codeine with 1 drop of Froehde's reagent. An intense blue color finally appears.

6. Marquis'[1] **Test.**—Concentrated sulphuric acid containing formalin dissolves codeine with a reddish violet color. This soon

[1] See preparation of reagents, page 642.

changes to blue-violet which persists for quite a long time. The spectrum of this solution shows absorption of orange and yellow.

7. Mecke's[1] Test.—Concentrated sulphuric acid containing selenious acid dissolves codeine with blue color quickly changing to emerald-green and finally becoming permanent olive-green. Gentle heat will produce a steel-blue color.

8. Furfural[2] Test.—Dissolve codeine in a little concentrated sulphuric acid and warm very gently with a drop of cane-sugar solution which must not be in excess. This will produce a purple-red color. This test may also be made by mixing a drop of sugar solution with codeine, dissolved in about 5 drops of 50–60 per cent. aqueous chloral hydrate solution, and then adding 1–2 cc. of concentrated sulphuric acid as an under layer. A carmine-red ring will appear at the zone of contact. The color is quite permanent and increases in intensity upon standing. If the sulphuric acid and chloral hydrate are thoroughly mixed, the entire liquid will be red. After a time the shade of color will be more of a red-brown.

Of the general alkaloidal reagents, iodo-potassium iodide, potassium bismuthous iodide, potassium mercuric iodide, and phosphomolybdic acid precipitate codeine even from very dilute solutions. Tannic acid, picric acid, gold and platinum chloride on the other hand are not as delicate.

Differences between Codeine and Morphine.—Morphine is soluble in solutions of caustic alkalies; codeine is not. The latter can be completely extracted by ether from an aqueous alkaline solution; morphine cannot, or at most only in traces. Codeine does not set iodine free from iodic acid, nor give with a mixture of ferric chloride and potassium ferricyanide a blue color, or blue precipitate. Solutions of neutral codeine salts are not colored blue by ferric chloride.

A cadaveric codeine has been described in the literature but it failed to give Pellagri's test, so characteristic of morphine and its derivatives. Therefore any possibility of confusing it with codeine is excluded.

NARCOTINE

Narcotine, $C_{22}H_{23}NO_7 = C_{19}H_{14}NO_4(OCH_3)_3$, is a component of opium, occurring to the extent of 4–8 per cent. and principally as the free alkaloid. In smaller quantity it appears to be present also in the officinal Capita papaveris.

[1] See preparation of reagents, page 643.

[2] This test depends upon furfural formed by the action of concentrated sulphuric acid upon cane-sugar. Very dilute aqueous furfural solution (1:1000) may be substituted for cane-sugar. Excess of furfural unlike cane-sugar does not interfere with the test. Tr.

Properties.—Narcotine crystallizes from alcohol in shining prisms, or in tufts of long needles, that are nearly insoluble in cold and but slightly soluble in boiling water. It dissolves most easily in chloroform and boiling alcohol. From the latter solution it crystallizes almost completely when cold. At 15° narcotine dissolves in 170 parts of ether, 31 parts of acetic ether, and 22 parts of benzene. Solutions of narcotine are neither alkaline nor bitter. In these respects narcotine is very different from the other opium alkaloids, as well as from the various other plant bases. Narcotine combines with one equivalent of acid, forming salts that usually do not crystallize. Their aqueous solutions have an acid reaction and they are readily soluble in alcohol. Narcotine salts of the weaker acids are decomposed by much water with separation of the free base; and salts of volatile acids behave in the same manner when their solutions are evaporated. With very weak acids, such as acetic acid, narcotine does not form salts. Consequently sodium acetate precipitates narcotine from solutions of other narcotine salts:

$$C_{22}H_{23}NO_7.HCl + CH_3.COONa = C_{22}H_{23}NO_7 + NaCl + CH_3.COOH.$$

Of the opium alkaloids known, sodium acetate not only precipitates narcotine from solutions of its salts but also papaverine and narceine. It does not, however, precipitate morphine, codeine and thebaine.

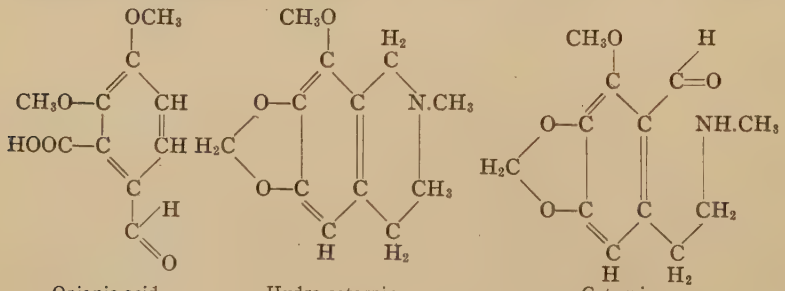
Constitution.—Narcotine is a tertiary base, for it combines with methyl iodide and other alkyl iodides forming ammonium iodides. Since by treatment with concentrated hydriodic acid, according to the method of Zeisel, three methyl-groups are split out as methyl iodide, the narcotine molecule must contain three methoxyl-groups. By hydrolytic cleavage, that is, by heating at 140° with water, with dilute sulphuric acid or barium hydroxide solution, narcotine yields nitrogen-free opianic acid and basic hydro-cotarnine which contains nitrogen:

$$\underset{\text{Narcotine}}{C_{22}H_{23}NO_7} + H_2O = \underset{\text{Opianic acid}}{C_{10}H_{10}O_5} + \underset{\text{Hydro-cotarnine}}{C_{12}H_{15}NO_3}.$$

By oxidative cleavage, that is, by treatment of narcotine with such oxidizing agents as nitric acid, manganese dioxide and sulphuric acid, lead dioxide, and ferric chloride, the products opianic acid and cotarnine are obtained:

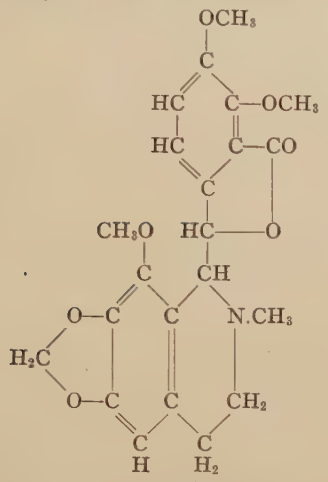
$$\underset{\text{Narcotine}}{C_{22}H_{23}NO_7} + H_2O + O_2 = \underset{\text{Opianic acid}}{C_{10}H_{10}O_5} + \underset{\text{Cotarnine}}{C_{12}H_{15}NO_4}.$$

These cleavage-products clearly show that narcotine is made up of two complexes, one nitrogen-free and the other containing nitrogen. The chemical constitution of these cleavage-products has been determined and is expressed by the following formulae:

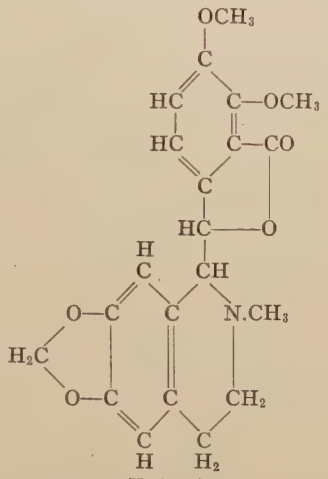


Opianic acid Hydro-cotarnine Cotarnine

Upon the basis of their experimental results, Roser and Freund have proposed the following structural formula for narcotine which they consider as definitely determined. Narcotine is entirely analogous to hydrastine, being in fact a methoxyl-derivative of the latter:

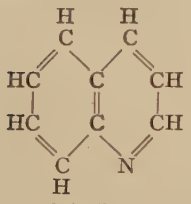


Narcotine

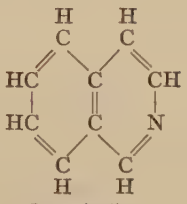


Hydrastine

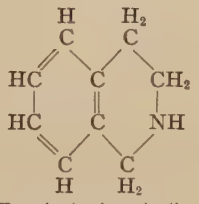
These two alkaloids are therefore iso-quinoline derivatives, that is, of tetrahydro-isoquinoline:



Quinoline



Iso-quinoline



Tetrahydro-isoquinoline

Physiological Action.—Narcotine possesses only very feeble narcotic properties. Administered internally, it exerts a sedative action upon the movements of the intestines. Injected into the blood, narcotine weakens the heart and therefore lowers the blood-pressure. Together with its character as a weak sedative, narcotine like morphine exhibits a stronger stimulating action upon the central nervous system. According to Straub,[1] narcotine makes morphine more potent in its narcotic and toxic action. At the same time it produces a qualitative change, for the respiratory center is less narcotized and thus regulation of the ventilation of the body is kept more intact. The practical advantage of opium over morphine is probably due as a matter of fact to the narcotine it contains. According to Meissner,[2] morphine stimulates even the respiratory center

[1] W. Straub: Pharmacodynamic Action of Narcotine in Opium. Biochem. Zeitschr. 41 (1912), 419.

[2] Meissner: Biochemisch. Zeitschr. 54 (1913), 395.

and from this point of view narcotine therefore may exert an influence antagonistic to the action of morphine. The inhibiting action of morphine upon intestinal peristalsis is not increased when combined with narcotine but is rather lessened (Trendelenburg[1]). An intensification by the other opium alkaloids of the action of morphine as an anodyne is due principally to narcotine. The greatest increase is produced by the combination of a molecule each of morphine and narcotine with the dibasic meconic acid, a preparation introduced into therapy by Straub[2] under the name of narcophine. The morphine-content in 0.03 gram of narcophine is 0.01 gram. This preparation is soluble in water. In consequence of rather slow absorption, narcophine exhibits its action more slowly than do morphine salts but it lasts longer for that reason.

Narcotine is eliminated from the human body in part by the intestinal glands and in part by the kidneys in the urine. But according to Dragendorff it may also be detected in the stomach, liver and spleen.

Detection of Narcotine

Because of its weak basic character, narcotine can be extracted almost completely by chloroform from the aqueous tartaric acid solution, whereas ether removes only traces. This behavior differentiates narcotine from most of the other plant bases. Obviously narcotine passes from the alkaline solution into ether and chloroform. As it comes from its ether solution, this alkaloid forms a varnish-like mass having scarcely any color and hardening after a time to a mass of radiating crystals. Narcotine is precipitated from its hydrochloric or sulphuric acid solution by iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, and phospho-molybdic acid even in dilutions of 1:5000.

Special Reactions

1. **Sulphuric Acid Test.**—Narcotine dissolves with stirring in concentrated sulphuric acid, producing a greenish yellow color that gradually changes to red-yellow and finally after several days to raspberry-red.

2. **Dragendorff's Test.**—A solution of narcotine in dilute sulphuric acid (1:5), evaporated upon the water-bath in a porcelain dish, or over a very small flame, has a red-yellow color, changing with stronger heat to crimson-red. As the acid begins to evaporate, blue-violet streaks radiate from the margin and finally the entire liquid has a dirty red-violet color. The same color-changes appear, if the yellow to red-yellow solution of narcotine in concentrated sulphuric acid is heated with extreme care over a very small flame.

3. **Froehde's Test.**—This reagent dissolves narcotine with blue-green to green color. If the reagent is more concentrated, the green color immediately changes to fine cherry-red, especially upon application of gentle heat. This color is quite persistent.

4. **Mecke's Test.**—Concentrated sulphuric acid containing selenious acid dissolves narcotine with steel-blue color that becomes cherry-red upon gentle warming.

[1] P. Trendelenburg: Archiv. f. exper. Path. u. Pharmakol. 81 (1917), 116.

[2] W. Straub: Münch. med. Wochenschr. 1912, 1542.

5. **Couerbe's Test.**—Dissolve narcotine in cold concentrated sulphuric acid and mix a trace of nitric acid with this solution after 1–2 hours. A red color will appear and gradually become more and more pronounced. Erdmann's reagent gives the same color-change. Concentrated sulphuric acid, containing 1 drop of ferric chloride solution in 10 cc., gives with narcotine upon warming a fine cherry-red color.

6. **Wangerin's[1] Test.**—Place a mixture of 0.01 gram of narcotine with 20 drops of pure concentrated sulphuric acid, and 1–2 drops of 1 per cent. cane-sugar solution upon a watch-glass and heat upon the water-bath with stirring for about 1 minute. At first the solution has a greenish yellow color that passes through yellow, yellow-brown, brown and brown-violet into a very beautiful and intense, pure blue-violet. In the beginning the intensity of this color increases somewhat in the cold, then fades and becomes discolored.

Applied to apomorphine, atropine, brucine, quinine, codeine, caffeine, hydrastine, morphine, physostigmine, pilocarpine, and strychnine, this test gives solutions that are colorless or nearly so. Only the morphine solution after a while has a pale pink color. Coniine and narcotine have a light yellow color; narceine chestnut-brown; and picrotoxin salmon-color to pale pink. Colchicin, digitalin and veratrine behave toward this reagent as toward pure concentrated sulphuric acid without the addition of the small quantity of sugar.

In this test 1–2 drops of 1 per cent. aqueous furfural solution may be substituted for the sugar solution. From yellow, brown, olive and other colors there finally emerges a deep, clear, dark blue. The brilliancy of this color increases somewhat upon standing. Gradually during a period of a few hours there is a change to a pure green color. For the detection of traces of narcotine (0.001 gram) use a 1 per cent. sugar solution.

HYDRASTINE

Hydrastine, $C_{21}H_{21}NO_2$, together with berberine, $C_{20}H_{17}NO_4$ (4 per cent.) and canadine, $C_{20}H_{21}NO_4$, occurs to the extent upon the average of 1.5 per cent. in hydrastis root, the root of Hydrastis canadensis, almost exclusively as the free alkaloid. The fluid extract prepared from this root and used in medicine should contain according to the German Pharmacopoeia at least 2 per cent. of hydrastine.

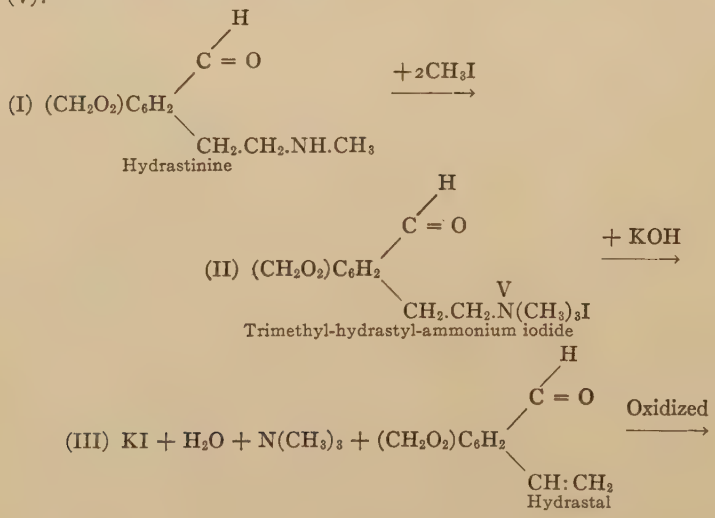
Preparation.—Extract hydrastis root with hot water containing acetic acid. Filter the extract, evaporate to a thin solution, and add dilute sulphuric acid in strong excess (about 3 volumes). Nearly all the berberine separates out in fine yellow crystals as acid sulphate, $C_{20}H_{17}NO_4.H_2SO_4$, whereas hydrastine remains in solution. Precipitate hydrastine from the filtered mother-liquor by means of ammonium hydroxide solution and purify the alkaloid by repeated crystallization from acetic ether or alcohol.

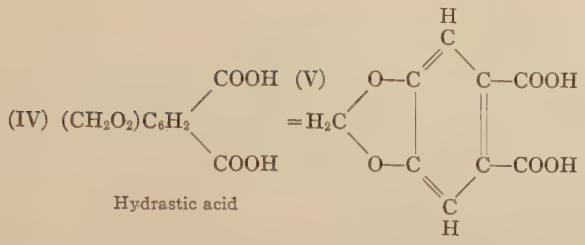
[1] A. Wangerin: Color Reactions of Narceine and Narcotine. Pharm. Ztg. 48 (1903), 607.

Properties and Constitution.—Hydrastine crystallizes from alcohol in lustrous, white, rhombic prisms melting at 132° and having a bitter taste. It is nearly insoluble in water, difficultly soluble in cold alcohol but freely soluble in hot alcohol, chloroform and benzene. Ether dissolves hydrastine less easily but still sufficiently so that this solvent may be used to extract the free base from an aqueous alkaline solution. Chloroform will extract hydrastine even from the aqueous tartaric acid solution. In chloroform solution this alkaloid is laevo-rotatory, whereas in dilute hydrochloric acid it is dextro-rotatory. Hydrastine is a weak, monacid base, its salts crystallizing as a rule with difficulty. Hydrastine hydrochloride, $C_{21}H_{21}NO_6.HCl$, obtained by passing hydrochloric acid gas into a solution of hydrastine in absolute ether, forms a white crystalline powder soluble in water. Formation at 100° from its constituents of hydrastine iodomethylate, $C_{21}H_{21}NO_6.CH_3I$, shows that hydrastine is a tertiary base. Heated according to the method Zeisel with hydriodic acid, hydrastine yields 2 molecules of methyl iodide, indicating the presence of 2 methoxyl-groups. Hydrastine undergoes oxidative cleavage with dilute nitric acid, yielding the nitrogenous base, hydrastinine, and nitrogen-free opianic acid:

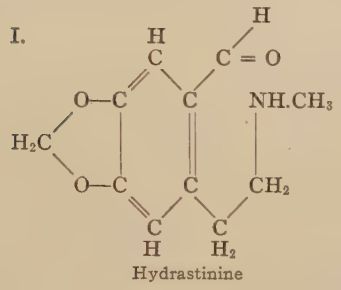
$$\underset{\text{Hydrastine}}{C_{21}H_{21}NO_6} + H_2O + O = \underset{\text{Hydrastinine}}{C_{11}H_{13}NO_3} + \underset{\text{Opianic acid}}{C_{10}H_{10}O_5}$$

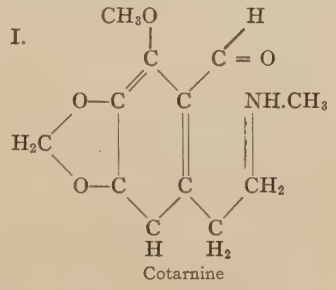
Since the constitution of opianic acid has long been known (see Narcotine, page 203), the only problem is the explanation of the nature of hydrastinine which has been determined, as well as that of many other alkaloids, by A. W. Hofmann's method of exhaustive methylation. Hydrastinine (I) is a secondary base which forms, when heated with an excess of methyl iodide, hydrastinine hydriodide and trimethyl-hydrastyl-ammonium iodide (II). Heated with alkalies, this ammonium iodide is decomposed into trimethylamine, hydriodic acid, and nitrogen-free hydrastal (III). The later upon oxidation gives hydrastic acid (IV) which was identified as the methylene ether of nor-meta-hemipinic acid (V):

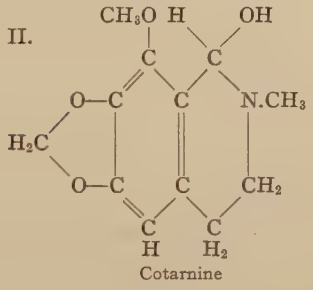




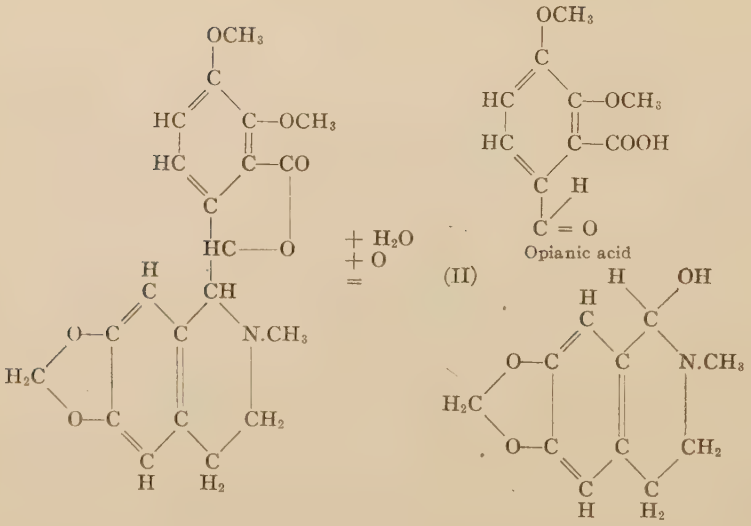
From these and other relations, it has been determined that cotarnine is a methoxy-hydrastinine. Both are able to react tautomerically:







Similarly narcotine is a methoxy-hydrastine. On the basis of the structural formulae on page 203 the oxidative cleavage of hydrastine into opianic acid and hydrastinine takes place in the following manner:



Hydrastine

Hydrastinine
(or formula I of the two tautomeric forms above)

Physiological Action.—According to R. Kobert, even small doses of hydrastine have a stimulating action upon the centers of the medulla oblongata in man and animals. Therefore it causes slowing of the pulse, increase in blood-pressure, and quickening of respiration. When the dose is increased, stimulation of these centers is changed to paralysis. Stimulation of the spinal cord accompanies the action upon the brain and may give rise to convulsions. Independent of this central action upon the nervous system, hydrastine causes muscular weakness of the heart. The smallest doses capable of producing an effect upon man give rise to increased blood-pressure. Hydrastinine on the other hand is not toxic at all for the muscles of the heart but rather increases the activity of the heart. Very small doses raise in man the tonus of the blood-vessels, especially when they are abnormally inactive. Large doses paralyze the blood-vessels and the heart-vagus after previous stimulation and cause death by paralyzing respiration. Hydrastinine has a stimulating action upon the isolated surviving uterus of many animals, strengthening its normal movements and in larger doses occasioning tetanus. Cotarnine, the nitrogenous cleavage-product of narcotine, acts upon the uterus like hydrastinine but has no action whatever upon the blood-vessels. Preparations of hydrastis and cotarnine are used to control abnormal flow of blood at menstruation.

Detection of Hydrastine

1. **Concentrated Sulphuric Acid.**—This acid dissolves hydrastine without color but the solution becomes violet when gently warmed.

2. **Froehde's Test.**—This reagent dissolves hydrastine with green color that gradually changes to brown.

3. **Mandelin's Test.**—Sulphuric acid containing vanadic acid dissolves hydrastine with rose-red color, immediately changing to orange-red and gradually fading.

4. **Fluorescence Test.**—Hydrastine dissolved in dilute sulphuric acid gives a colorless solution that does not fluoresce (distinction from quinine). Add very dilute potassium permanganate solution drop by drop, shaking well after each addition. Hydrastinine is formed and the solution exhibits beautiful blue fluorescence.

Ether extract of the aqueous alkaline solution, obtained by the Stas-Otto method, usually leaves hydrastine in a crystalline condition.

QUININE

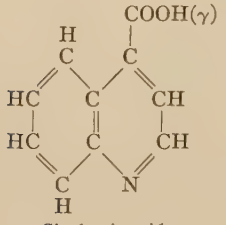
Quinine, $C_{20}H_{24}N_2O_2$, occurs together with cinchonine and other alkaloids as salts of quinic and quinotannic acid, especially in the barks of various species of Cinchona. These various barks show a very wide range in the amount of quinine they contain. The South American barks rarely contain more than 5 per cent. and frequently only 2–3 per cent. of quinine, whereas the bark of Calisaya Ledgeriana from Java has repeatedly been found to contain as high as 13 per cent.

Properties.—Addition of ammonia to the solution of a quinine salt first produces a white, cheesy, amorphous precipitate of anhydrous quinine. After a short time, however, it takes up 3 molecules of water and changes to crystalline quinine hydrate, $C_{20}H_{24}N_2O_2.3H_2O$. This compound may be obtained in long, silky needles by crystallization from dilute alcohol, or if a solution of quinine in strong ammonia is saturated in sealed tube at 100° and allowed to cool slowly. Anhydrous quinine separates from solution of quinine hydrate in strong alcohol, ether and benzene. The former may be obtained in the form of silky needles by dissolving quinine hydrate in hot dilute alcohol and allowing the solution to stand for a long time at 30°. Quinine hydrate melts at 57°. Heated at higher temperature, it solidifies again and then melts at 171°, the melting-point of anhydrous quinine. Anhydrous quinine dissolves at 15° in 1960 parts of water and the hydrate in 1670 parts and in about 900 parts of boiling water. The solubility of this base in water is increased by addition of ammonia but decreased by sodium or potassium hydroxide solution. Alcohol, ether, benzene and carbon disulphide are good solvents of quinine. Quinine solutions are laevo-rotatory, the degree of rotation varying considerably according to concentration and solvent. Alcoholic or aqueous solutions of quinine salts, prepared by means of sulphuric, nitric, phosphoric, formic, acetic, tartaric or citric acid, exhibit fine blue fluorescence. But fluorescence does not appear with hydrochloric, hydrobromic and hydriodic acid. In fact addition of one of these acids to solutions of the other salts even inhibits fluorescence. Chlorides, bromides and iodides

have the same effect. Concentrated sulphuric acid dissolves quinine giving a colorless or at most a pale yellowish color.

Constitution.—Quinine is a diacid, ditertiary base, combining with one and two equivalents of acid and forming salts that usually crystallize well. The salts with one equivalent of acid are the more stable. Quinine hydrochloride, $C_{20}H_{24}N_2O_2.HCl.2H_2O$, used in medicine, crystallizes in long, delicate tufts of needles. Quinine sulphate, the officinal quininum sulphuricum, $(C_{20}H_{24}N_2O_2)_2$-$H_2SO_4.8H_2O$, forms white silky needles. Aqueous and alcoholic solutions of these two salts have a neutral reaction, whereas solutions of the so-called acid quinine hydrochloride, $C_{20}H_{24}N_2O_2.2HCl$, and acid quinine sulphate, or quinine bisulphate, $C_{20}H_{24}N_2O_2.H_2SO_4.7H_2O$, have a strong acid reaction.

In accordance with its ditertiary character, quinine can combine with 2 molecules of an alkyl iodide, for example, it forms with methyl iodide quinine di-iodo-methylate, $C_{20}H_{24}N_2O_2.2CH_3I$. The quinine molecule also contains a hydroxyl-group, for it gives a monacetyl and a monobenzoyl derivative. The presence of one methoxyl-group may also be detected by Zeisel's method. Therefore the empirical formula of quinine may be resolved into $C_{19}H_{20}(OH)(OCH_3)N_2$. The empirical formula of quinine, $C_{20}H_{24}N_2O_2$, differs from that of cinchonine, $C_{19}H_{22}N_2O$, by (CH_2O). Consequently quinine must be regarded as a methoxy-cinchonine, for the methoxyl-group cannot be detected in the cinchonine molecule. Upon oxidation with chromic acid, cinchonine gives cinchonic acid, identical with quinoline-γ-carboxylic acid, and quinine under the same conditions gives quinic acid, or p-methoxy-cinchonic acid:



Cinchonic acid

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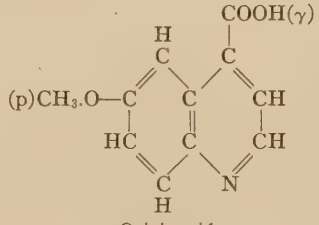
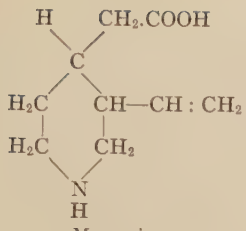
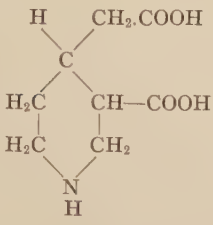


Quinic acid

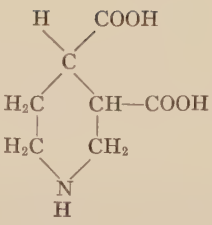
Both alkaloids upon oxidation also give nitrogenous compounds, meroquinene, cincho-loiponic acid and loiponic acid. Consequently there is no doubt that cinchonine and quinine contain two nitrogenous nuclei, one of which is a quinoline complex. The second nucleus is connected with the latter in the γ-position, as shown by formation of cinchonic and quinic acid. Meroquinene, cincho-loiponic acid and loiponic acid, derived by oxidation with chromic acid from the so-called "second half" of the cinchonine and quinine molecule, form a continuous series of oxidation products, since meroquinene can be oxidized to cincho-loiponic acid and the latter to loiponic acid. The following formulae offer the most satisfactory explanation of the relationship existing between these three compounds:



Meroquinene



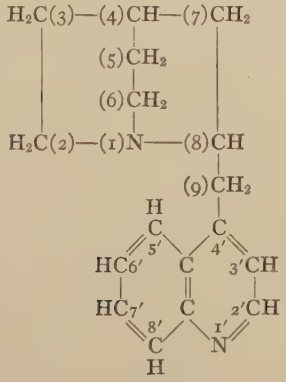
Cincho-loiponic acid



Loiponic acid

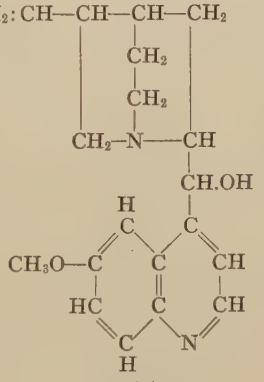
Rabe,[1] whose researches in the field of the cinchona alkaloids have met with success, has recently introduced the name "rubane" for the mother-substance from which quinine and related alkaloids are derived. Cinchona alkaloids have thus far been found only in plants belonging to the family of the Rubiaceae. This mother-substance, rubane, is a disubstituted methane and consequently is designated as (quinuclidyl-8)-(quinolyl-4′). The position of substituents in the quinoline nucleus is designated in the usual manner by α, β, γ, ortho, etc., or better by the primed numerals 1′, 2′, 3′, etc., and in the other rings by the number 1–9, beginning with the nitrogen atom of the quinuclidine nucleus and ending with the C-atom attached to the quinoline nucleus in the γ-position, as shown in formula I, that of the mother-substance, rubane. Quinine, formula II, in accordance with this nomenclature would be 6′-methoxy-3-vinyl-rubanol-(9) and quinotoxine, formula III, easily derived from it as 6′-methoxy-3-vinyl-rubatoxanan-(9). This representative of the cinchona-toxines was earlier called according to Pasteur "Quinicine" which was later changed by v. Miller and Rhode to quinotoxine.

I.



Rubane

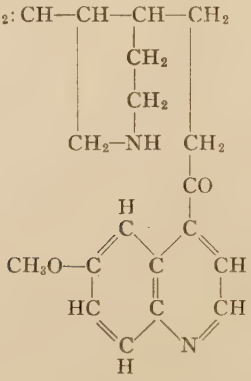
II.



Quinine

[1] P. Rabe: Contribution to the Knowledge of Cinchona Alkaloids, XXIII: Nomenclature and Phenomena of Isomerism. Ber. d. Deutsch. chem. Ges. 55 (1922), 922.

III.



Quinotoxine

Physiological Action.—Quinine is a pronounced protoplasmic poison and in sufficiently large doses paralyzes and destroys not only different unicellular organisms, such as plasmodia, but also ciliated cells, white and red blood-corpuscles, muscle cells, nerve cells, plant cells and enzymes. The partition process of the cell nucleus is deranged by quinine (O. Hertwig). Bacteria and molds are paralyzed only by relatively large doses, and under the same conditions fermentations are unfavorably influenced. Schmiedeberg assumes that quinine in rather small doses increases the vitality and heightens the capacity of the particular cells in their functions. Quinine diminishes frequency of heart-beat even in presence of atropine. The blood-vessels of isolated surviving organs are greatly dilated by quinine (Kobert). For this reason and also because of paralysis of vaso-motor centers, blood-pressure drops. As a result of dilatation of cutaneous blood-vessels there is increased loss of heat. It is not impossible that oxidation ferments, which control processes of combustion in the tissues, are also concerned in lowering of efficiency. The so-called quinine-drunk is probably due to lowering of the efficiency of the cortex of the brain. It manifests itself as heavy stupor of the head. In the case of individual animal species death ensues during paralysis of the respiratory center accompanied by great weakness of heart action. In man convulsions may appear in quinine coma (Kobert).

The symptoms of quinine poisoning are: abdominal pains, emesis, diarrhoea, ringing in the ears, difficulty in hearing, deafness, permanent blindness, disturbances of speech, paralysis of the limbs, chills, cold sweats, and severe collapse lasting several days. There is also swelling of the face and tongue, and skin eruptions (quinine exanthema) are somewhat frequent even after rather small doses. Haemoglobinuria, methaemoglobinuria (black water fever), icterus, vomiting of blood, bloody diarrhoea, albuminuria lasting for weeks, fever, and cyanosis have been observed. A fatal case of poisoning by quinine, observed by Strzyzowski and Petroff, is described in the literature. A woman 22 years old with suicidal intent took 15 grams of quinine sulphate and drank the juice of two lemons which facilitated solution of the quinine salt. After about 2 hours she felt very weak, had ringing in the ears, dizziness, emesis and looked pale.

After 4 hours she was wholly unconscious and had convulsions. The pulse was small but regular; respiration normal. After 5 hours severe convulsions, action of the heart weak and irregular, pupils unreactive, extremities cool; repeated emesis. After 6 hours continued convulsions during which death ensued. In cases followed by recovery, derangement of sight and hearing on both sides persisted for weeks or months or for life.

Quinine hydrochloride is absorbed from all body cavities, from subcutaneous tissues and from the gastro-intestinal tract. After use of quinine, metabolism is at first increased for a short time and then considerably diminished. As a result elimination of nitrogen drops.

Elimination in Man.—Quinine, administered internally in daily doses of 0.5 gram of quinine hydrochloride, is eliminated for the most part unchanged in the urine. Schmitz[1] was unable to find in the urine transformation products of quinine, such as dihydroxy-quinine described by Kerner. Within 48 hours 20–30 per cent. of quinine, taken by the mouth in the form of powdered hydrochloride, was eliminated. After continued administration of quinine, the quantity eliminated daily in the urine varied between 19 and 35 per cent., averaging about 26 per cent. Elimination of quinine in urine appears to be less when used subcutaneously than when taken by the mouth, averaging daily only 16 per cent. That portion of quinine not appearing in the urine is apparently destroyed in the organism. After prolonged use of quinine, the power of the human body to destroy this alkaloid does not seem to increase, according to results of experiments made by Schmitz. Details with regard to detection and quantitative estimation of quinine in urine are given in Chapter V (see page 552).

Traces of quinine may also be found in saliva, perspiration and milk. In animals, after subcutaneous injection of considerable doses, a large part of the quinine is eliminated in the gastro-intestinal tract and appears in the faeces scarcely altered in form.

In the few cases, ending acutely in death, the lethal dose amounted to 8–15 grams of quinine hydrochloride; but in one case recovery followed after 15 grams of this quinine salt and in another after even 20 grams.

Detection of Quinine

Ether, benzene or chloroform extracts quinine from aqueous alkaline solution. Upon evaporation, ether deposits the alkaloid as a resinous, amorphous varnish in which its presence may be recognized by the following tests:

1. Fluorescence Test.—Dilute sulphuric acid dissolves the residue from ether solution and produces a fine blue fluorescence if quinine is present. Even in a dilution of 1:100,000 this fluorescence is distinctly visible.

[1] R. Schmitz: Elimination of Quinine in Human Urine. Arch. f. exper. Path. u. Pharmak. 56 (1907), 301.

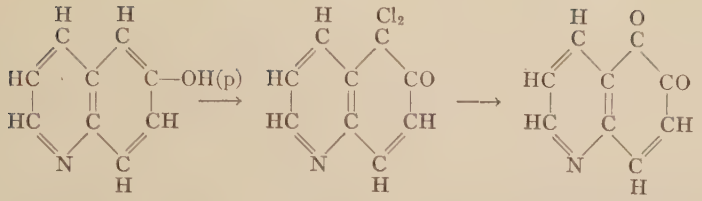
2. Thalleioquin Test.—Dissolve the residue from the ether solution in a little dilute acetic acid and add 5–10 drops of saturated chlorine water. The colorless solution has a faint blue fluorescence and will at once give a fine green color upon addition of ammonia in excess, if quinine is present. Larger quantites of quinine give a green precipitate, thalleioquin. It is always obtained as an amorphous substance of variable composition. Thalleioquin is soluble in alcohol and chloroform but insoluble in ether. E. Pollacci heats quinine (about 0.01 gram) gradually to boiling with 2–3 cc. of water, a little lead dioxide, and 2 drops of dilute sulphuric acid. The clear solution after settling is decanted or filtered and carefully covered with 5–6 drops of ammonia. A green zone will appear at the contact-surface of the two liquids.

According to Abensour the thalleioquin test may be made more easily and with greater certainty, if carried on in a neutral or only faintly acid alcoholic solution and if the dyestuff is dissolved in a little chloroform. Add to 10 cc. of the solution to be tested saturated bromine water drop by drop until the fluorescence has disappeared. Then dilute the mixture with the same volume of alcohol and add 1–2 drops of ammonia. The green dyestuff may also be taken up in chloroform. When the material is a quinine salt, dissolve in a little alcohol, add an equal volume of water, and proceed as described above.

Interferences with the Thalleioquin Test.—Antipyrine interferes with this test. Mixtures of 1 per cent. solutions of antipyrine and quinine give finally a fine red instead of a green color. This interference does not cease until these two substances are in the proportion of 0.25 part of antipyrine to 5 parts of quinine. Caffeine also interferes with the thalleioquin test, when the proportion is 2 parts of quinine to 3 parts of caffeine. Other compounds, such as urea, prevent appearance of this color, whereas atropine, codeine, cocaine, morphine, pilocarpine, strychnine, as well as carbolic acid and chloral hydrate, have no effect upon the thalleioquin test.

Fühner[1] has shown that the thalleioquin reaction is connected with the p-oxy-quinoline complex. Chlorine passed into a solution of pure p-oxy-quinoline hydrochloride cooled with ice produces a white crystalline precipitate. This substance crystallizes from petroleum ether in colorless prisms or plates melting at 58°. Structurally it is 5,5-dichloro-6-keto-quinoline. Solutions of this dichloro-keto-quinoline and of its hydrochloride are colored pure green or blue by ammonia. Fühner thinks 5,6-quinoline-quinone is probably formed and gives the green color with ammonia:

[1] H. Fühner: Thalleioquin Reaction of Quinine and the Kynurenic Acid Reaction of Jaffé. Ber. d. Deutsch. chem. Ges. 38 (1905), 2713.



3. Herapathite Test.—Mix 30 drops of acetic acid, 20 drops of absolute alcohol, and 1 drop of dilute sulphuric acid (20 per cent. H_2SO_4). Add 20 drops of this mixture to 0.01 gram of quinine and heat to boiling. Finally add 1 drop of alcoholic solution of iodine (1:10), or 2 drops of 0.1 n-iodine solution. At once, or sometimes not until the solution has stood for some time, green leaflets having a metallic lustre separate. This product is so-called "herapathite," an iodine compound of quinine having the constant composition, $4C_{20}H_{24}N_2O_2.3H_2SO_4.2HI.4I.3H_2O$. It may be recrystallized from boiling alcohol. Herapathite crystals are pale olive-green by transmitted light but by reflected light have a fine, cantharidin-green, metallic lustre. They have a strong polarizing action upon light.

Caustic alkalies, ammonia, sulphurous acid and hydrogen sulphide decompose herapathite. A. Christensen recommends keeping on hand the following reagent for the herapathite test: 1 part of iodine; 1 part of 50 per cent. hydriodic acid; 0.8 part of sulphuric acid; and 50 parts of 70 per cent. alcohol. Add a few drops of this reagent to the alcoholic solution to be tested for quinine.

4. Abensour's[1] Erythroquinine Test.—Add 1 drop each of half-saturated bromine water, potassium ferrocyanide solution (1:10), and ammonia (10 per cent. NH_3) to 10 cc. of a faintly acid, best with acetic acid, solution of quinine. This mixture, if shaken, gradually turns red. This test is given especially well, if the mixture is at once shaken with chloroform. This solvent takes up the color and has the appearance of a chloroform solution of iodine. This is an exceedingly delicate test for quinine. Chloroform takes on a brilliant red-violet color even with 1 cc. of a 1:100,000 quinine solution = 0.01 mg. of alkaloid.

Of the alkaloidal reagents, potassium bismuthous iodide is especially recommended as a precipitant of quinine. With quinine sulphate solutions, this reagent produces precipitates having an intense

[1] J. Abensour: Detection of Quinine. Journ. de Chim. et Pharm. (6) 26 (1907), 25.

yellow-red color. Shaken with sodium hydroxide solution, this precipitate is decomposed and unaltered quinine may be obtained by extraction with ether and evaporation of the ether solution. Thoms[1] has made use of this reaction in the quantitative separation of quinine from mixtures.

CAFFEINE

Since caffeine is a weak base, ether extracts only a little of this alkaloid from tartaric acid solution. The greater part is extracted by ether, or better chloroform, from aqueous alkaline solution. Ether usually deposits caffeine in white, shining needles arranged in clusters. Since caffeine is rather difficultly soluble in ether, the aqueous alkaline solution should be repeatedly extracted with large quantities of this solvent. For tests characteristic of this alkaloid see page 148.

ANTIPYRINE

In the Stas-Otto process most of the antipyrine is extracted by ether from aqueous alkaline solution. It is usually purer from alkaline than from acid solution and frequently appears in crystalline leaflets. Antipyrine differs from most alkaloids in having only a faintly bitter taste and in being freely soluble in water. To identify antipyrine, dissolve the ether residue in a little water and divide the solution into two equal parts. Test one portion with ferric chloride solution and the other with fuming nitric acid. Further details with regard to tests for antipyrine will be found on page 142.

PYRAMIDONE

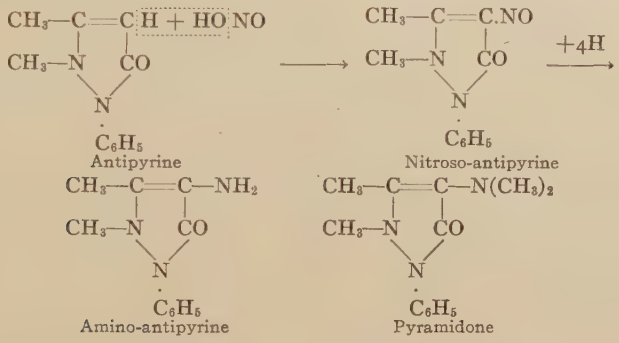
Pyramidone, or 4-dimethyl-amino-antipyrine, $C_{13}H_{17}N_3O$, has been extensively used of late as an antipyretic and anodyne. Its effects are like those of antipyrine but its action is 3–4 times stronger, so that a correspondingly smaller dose, that is, 0.25–0.3 gram, may be taken.

Properties.—Pyramidone is a white, crystalline powder having a faintly bitter taste and melting at 108°. It dissolves in about 30 parts of cold and more easily in hot water. It is freely soluble in alcohol (1:2), in chloroform (1:1), and somewhat less in ether (1:9). The aqueous solution of pyramidone has a faint alkaline

[1] D. Jonescu and H. Thoms: Precipitation and Quantitative Estimation of Alkaloids by Potassium Bismuthous Iodide. Ber. d. Deutsch. pharm. Ges. 16 (1906), 130.

reaction. Pyramidone is a stronger base than antipyrine and consequently only traces pass from tartaric acid solution into ether. It differs from antipyrine in being a strong reducing agent. Pyramidone, for example, will reduce gold chloride even in the cold, whereas antipyrine and tolypyrine require heat.

Preparation.—Antipyrine dissolved in concentrated acetic acid is converted by potassium nitrite into nitroso-antipyrine appearing as green crystals. This compound in alcoholic solution is reduced by zinc dust and acetic acid to amino-antipyrine. The latter, treated with methyl iodide and potassium hydroxide in methyl alcohol solution, is converted into dimethyl-amino-antipyrine = pyramidone:



Detection of Pyramidone

Pyramidone can be easily and completely extracted by ether or chloroform from aqueous alkaline solution. Upon evaporation of solvent it usually appears in fine needles. Pyramidone solutions give precipitates with most of the general alkaloidal reagents. Because of its strong reducing properties, it reacts especially easily with oxidation agents, such as ferric chloride, iodine and silver nitrate. Pyramidone may be identified by the following tests:

1. **Nitrous Acid Test.**—An aqueous pyramidone solution, acidified with dilute hydrochloric or sulphuric acid, exhibits a temporary blue-violet color with a little potassium nitrite solution. This color disappears gradually; rapidly upon further addition of potassium nitrite. This test is useful in detecting antipyrine in pyramidone, for the former gives quite a stable green color.

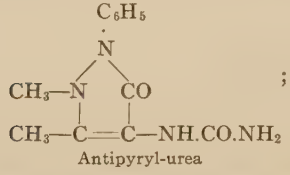
2. **Ferric Chloride Test.**—With ferric chloride solution aqueous pyramidone solutions give the same blue-violet color produced by iodine solution. This color at once passes into red-violet and soon disappears.

3. **Silver Nitrate Test.**—Aqueous pyramidone solutions, treated with silver nitrate solution, first have an intense blue color due to

formation of colloidal silver. After a while the liquid becomes dark and metallic silver separates.

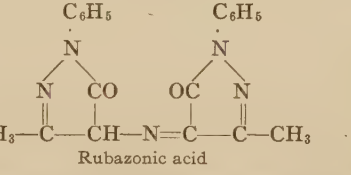
Pyramidone finds therapeutic use in the form of salts and molecular compounds, such as the salicylate, melting at 68–76°, and trigemine, a compound of pyramidone with butyl-chloralhydrate, melting at 85°.

Behavior in the Human Organism and Elimination.—Following internal administration of pyramidone, the urine is usually colored bright purplish red. It contains a conjugated glycuronic acid, rubazonic acid and antipyryl-urea. Even after large doses, unchanged pyramidone cannot be found in the urine. After standing for some time, pyamidone-urine frequently deposits rubazonic acid as a sediment, consisting of red needles soluble in ether or chloroform but especially in acetic ether. The solvent has a ruby-red color. If pyramidone-urine is acidified, rubazonic acid may be extracted with acetic ether. Formation in the human organism of rubazonic acid and antipyryl-urea from pyramidone is due to demethylation, as may be seen by comparing the formulae below with that of pyramidone given above. Therefore the same processes go on in the case of pyramidone as in that of the purine bases, caffeine, theobromine and theophylline, from which in the course of animal metabolism methyl-groups also attached to nitrogen undergo cleavage, at least in part.



Antipyryl-urea

;



Rubazonic acid

Detection of antipyryl-urea and rubazonic acid in pyramidone-urine, according to the method of Jaffé, is taken up in Chapter V of this book (see page 566).

Fatal poisonings from pyramidone are thus far unknown. In some instances this pyrazolone derivative has caused gastric disturbance and been responsible for such marked secretion of sweat that further administration of pyramidone had to be abandoned. (Meyer-Gottlieb.)

C. Examination of Ether and Chloroform Extracts of Ammoniacal Solution

(α) **Ether Extract.**—Apomorphine and traces of morphine.[1]

(β) **Chloroform Extract.**—Morphine and narceine. Antipyrine, colchicin and caffeine[2] may also be in this extract.

[1] Ether dissolves traces of freshly precipitated, amorphous morphine.

[2] Antipyrine, colchicin and caffeine, though freely soluble in chloroform, dissolve with difficulty in ether. If these substances are not completely extracted by ether from the aqueous alkaline solution, they will be present in the chloroform extract together with morphine and narceine. Colchicin in particular will be obtained at this point in a fairly pure condition.

The preliminary test for morphine and apomorphine is given on page 223.

The aqueous alkaline solution (see page 106), separated from ether, must be tested further for substances mentioned under α and β. Apomorphine may be recognized by the green color of the original aqueous tartaric acid solution. Excess of sodium hydroxide solution causes oxidation, especially if the solution is exposed for any time to air, and gradually changes the color to purple-red. Moreover, ether extracts, both of the acid and alkaline solutions, are red or violet-red when apomorphine is present. If the aqueous and ether solutions, obtained by the Stas-Otto method, do not have the characteristics mentioned, examination for apomorphine is unnecessary, that is, extraction with ether may be omitted. In that case proceed at once with the examination for morphine and narceine.

To extract apomorphine, morphine and narceine with the proper solvent, the aqueous solution separated from ether, which is alkaline from sodium hydroxide, should be rendered alkaline with ammonium hydroxide solution. First acidify the solution with dilute hydrochloric acid (test with blue litmus paper) and then add ammonium hydroxide solution until alkaline.

(α) If there is indication of apomorphine, extract the aqueous ammoniacal solution immediately with ether and, after removing the ether extract, repeatedly with hot chloroform.

(β) If there is no indication of apomorphine, extract the ammoniacal solution direct several times with hot chloroform (see below).

(α) Ether Extract

APOMORPHINE

Apomorphine is derived from morphine by loss of 1 molecule of water under the influence of hydrochloric, sulphuric or oxalic acid, zinc chloride, as well as the alkalies:

$$\underset{\text{Morphine}}{C_{17}H_{19}NO_3} = H_2O + \underset{\text{Apomorphine}}{C_{17}H_{17}NO_2}.$$

Codeine also gives apomorphine when heated at 140° with concentrated hydrochloric acid:

$$\underset{\text{Codeine}}{C_{17}H_{18}NO_2(OCH_3)} + HCl = CH_3Cl + H_2O + \underset{\text{Apomorphine}}{C_{17}H_{17}NO_2}.$$

Preparation.—Heat morphine (1 part) in sealed tube for about 3 hours at 140–150° with 25 per cent. hydrochloric acid (10 parts). Cool the contents of the tube, add sodium bicarbonate in slight excess, excluding air as much as possible,

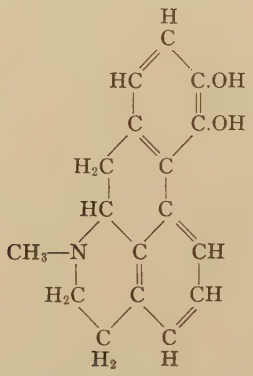
and immediately extract with ether or chloroform. Morphine, that has not undergone change, remains undissolved. Addition of a little concentrated hydrochloric acid to the ether or chloroform solution causes crystallization upon the walls of the container of apomorphine hydrochloride (Apomorphinum hydrochloricum) used in medicine. The free base apomorphine may be precipitated from this salt, after it has been purified by crystallization from a little hot water, by sodium bicarbonate:

$$C_{17}H_{17}NO_2.HCl + NaHCO_3 = C_{17}H_{17}NO_2 + NaCl + CO_2 + H_2O.$$

Properties.—Apomorphine freshly precipitated is white and amorphous. In contact with air in moist condition it turns green. It is somewhat soluble in water and dissolves easily in alcohol, ether and chloroform. Aqueous and alcoholic solutions of apomorphine, which are colorless when freshly prepared, turn green in the air as a result of oxidation. Aqueous and alcoholic solutions of apomorphine thus changed by oxidation are emerald-green. Ether and benzene solutions are purple-violet; those in chloroform blue-violet. As a phenol, apomorphine is readily soluble in sodium or potassium hydroxide solution. A solution of the alkaloid in potassium hydroxide solution absorbs oxygen from the air and turns brown. Apomorphine differs from morphine, especially in solubility in ether, benzene and cold chloroform. Morphine is almost insoluble in these solvents.

Constitution.—Apomorphine in a monacid, tertiary base and at the same time a di-acid phenol. As such it is easily soluble in solutions of caustic alkalies. The presence of two phenolic hydroxyl-groups is shown by the fact that it gives in alkaline solution with benzoyl chloride colorless, crystalline needles of dibenzoyl-apomorphine, $C_{17}H_{15}N(O.CO.C_6H_5)_2$, and with diazo-methane dimethyl-apomorphine, $C_{17}H_{15}N(O.CH_3)_2$.

Since dimethyl-apomorphine, after previous exhaustive methylation, gives an intermediate product that is oxidized by potassium permanganate to dimethoxy-phenanthrene carboxylic acid, apomorphine like morphine is to be regarded as a derivative of phenanthrene, $C_{14}H_{10}$ (Pschorr). Upon the basis of his experimental results, Pschorr[1] has proposed for apomorphine the accompanying structural formula.



[1] R. Pschorr: Problem of the Constitution of Apomorphine. Ber. d. Deutsch. chem. Ges. 39 (1906), 3124; and Ibid. 40 (1907), 1984.

Physiological Action.—Apomorphine frequently finds therapeutic use as an expectorant and emetic but not as a narcotic. In man after use of medicinal doses, emesis is the only evidence of central stimulation. In a case described in the literature, a man had taken 0.2 gram of apomorphine hydrochloride. He fainted repeatedly, had painful breathing and a feeling of suffocation until finally abundant emesis occurred and the pain disappeared. Intense dizziness, falling down, deathly pallor, cold sweats and rattling in the throat may appear (Loeb). According to Bergell and Pschorr, the emetic action of apomorphine is due to presence of the two hydroxyl-groups. Substances formed by esterifying or etherifying these groups are without emetic properties. It is a notable fact that different classes of animals do not behave in the same manner toward apomorphine. Dogs have emesis after 0.1 mg. of apomorphine and do not die even after 2 grams but are only narcotized and paralyzed for a rather long time. Apomorphine does not produce emesis at all in swine, cattle and horses but has a highly stimulating action. Many horses like many persons are specifically susceptible and die even after relatively small doses of apomorphine.

Detection of Apomorphine

Ether will not extract apomorphine from a solution containing tartaric acid but will dissolve its colored oxidation products. This solvent behaves similarly toward solutions of this alkaloid containing caustic alkalies. Ether or chloroform will extract apomorphine only from a solution alkaline with ammonium hydroxide or sodium bicarbonate. Ether solutions of apomorphine usually deposit a greenish, amorphous residue. This alkaloid has a strong reducing action. It will reduce iodic acid with liberation of iodine which may be detected by means of chloroform. It will produce a purple color with gold chloride due to metallic gold. Most of the tests that follow are the result of oxidation of apomorphine by which green, violet or blue oxidation products are formed. This is true of the tests with iodine, ferric chloride, potassium dichromate, potassium ferricyanide, or atmospheric oxygen.

1. **Sulphuric and Nitric Acids.**—Concentrated sulphuric acid dissolves apomorphine without color. Addition of a drop of concentrated nitric acid to this solution produces a fugitive violet color, changing at once to blood-red and finally to yellow-red. Concentrated nitric acid alone with apomorphine gives a violet-red solution that immediately becomes red-brown and brown-red.

2. **Pellagri's Test.**—Dissolve apomorphine in dilute hydrochloric acid and add sodium bicarbonate to faint alkaline reaction. Then add drop by drop 1–3 drops of dilute alcoholic iodine solution, shaking well after each addition. The color of the solution will be

blue-green or emerald-green. Extract with ether (2–3 cc.) and this solvent will have a fine violet-red color, whereas the aqueous solution will remain green.

3. Froehde's Test.—This reagent dissolves pure apomorphine with green color, or violet if the alkaloid has been altered by air.

4. Wangerin's[1] Test.—Prepare a fresh solution of apomorphine hydrochloride (about 1 per cent.). Add 4 drops of potassium dichromate solution (0.3 per cent.) to 1 cc. of this solution and shake for about 1 minute. The solution will be deep dark green. Then add a few cc. of acetic ether and shake again. This solvent will become violet. Now add about 5 drops of stannous chloride solution[2] (1 per cent.) and shake well. The color of the acetic ether layer will change to green and, upon further addition of a few drops of potassium dichromate solution, the acetic ether will again become violet. If 10 cc. of chloroform are substituted for acetic ether in this test, the oxidation products of apomorphine will impart the same violet color to the choloroform. But if stannous chloride solution is carefully added, the color will change to pure indigo-blue and persist when shaken again with potassium dichromate solution.

5. Schmidt's[3] Tests.—The following especially typical tests are recommended for detection of apomorphine hydrochloride:

(a) A drop of dilute ferric chloride solution (1:10) will turn 10 cc. of an aqueous apomorphine hydrochloride solution blue even in dilution of 1:10,000.

(b) Shake 10 cc. of the same apomorphine hydrochloride solution with 1 cc. of chloroform. Then render alkaline with sodium hydroxide solution and at once shake well with air. The aqueous solution acquires a fugitive violet color and the chloroform is blue.

6. Feinberg's[4] Test.—Dissolve apomorphine hydrochloride in not too little water, add 3 drops of 1 per cent. potassium ferricyanide solution, and shake well with 1 cc. of benzene. The color of the benzene layer will be amethyst. Addition of a few drops of dilute

[1] A. Wangerin: Helch's Pilocarpine Test and Apomorphine Reactions. Pharm. Ztg. 47 (1902), 599 and 739 to 740.

[2] Dissolve 1 gram of $SnCl_2.2H_2O$ in 50 cc. of pure 25 per cent. hydrochloric acid and bring the volume to 100 cc. when cold with pure dilute hydrochloric acid.

[3] E. Schmidt: Apomorphine Hydrochloride. Apotheker-Zeitung 23 (1908), 657.

[4] M. Feinberg: Contributions to the Knowledge of Apomorphine. Zeitschr f. physiol. Chem. 84 (1912), 363.

sodium carbonate solution produces with agitation first a violet-red that changes after long standing to a fine violet color. This is a very delicate test, capable of detecting 0.00003 gram of apomorphine in 1 cc. of solution, and morphine and other opium alkaloids cause no interference.

(β) Chloroform Extract

Preliminary Morphine Test.—As a preliminary test for morphine, acidify a small portion of the aqueous alkaline solution (see page 107), separated from ether with dilute sulphuric acid, add a few drops of iodic acid solution, and extract with a little chloroform. If the latter is violet from iodine, morphine may be present. But a final decision regarding presence of morphine should not be made from a positive test, since many other organic substances besides this alkaloid will reduce iodic acid.[1] This is a delicate preliminary test for morphine and that is its only value. If no reaction takes place, probably morphine is not present; if positive, morphine may be present. Apomorphine also reduces iodic acid.

To detect morphine and narceine with certainty, render the aqueous solution alkaline with ammonia, or sodium bicarbonate, and extract at once in a capacious flask as already directed (see page 107) with considerable hot chloroform.[2] Separate the two liquids as usual in a separating funnel. Several extractions of the aqueous solution with fresh portions of hot chloroform, or hot chloroform-alcohol mixture, are necessary because of the slight solubility of morphine even in boiling chloroform. Should the chloroform and aqueous solution form a troublesome emulsion that will not separate, add a few drops of alcohol, set the flask upon a warm but not boiling water-bath, and carefully rotate the flask from time to time. This procedure usually causes immediate separation of the two liquids. Place the combined chloroform extracts in a dry flask, add a few crystals of dry sodium chloride, or anhydrous sodium sulphate, to remove adherent water, pour the chloroform when clear through a dry filter, and evaporate in not too large a

[1] In testing animal matter containing absolutely no morphine, the author has repeatedly obtained extracts that strongly reduced iodic acid.

[2] C. Kippenberger (Contributions to the Analytical Chemistry of Alkaloids. Zeitschr. f. analyt. Chem. 39 (1900), 201, 290) extracts morphine with chloroform, containing 10 per cent. by volume of absolute alcohol. In several instances the author has found this chloroform-alcohol mixture very useful and prefers it at the present time to pure chloroform.

glass dish upon a warm water-bath. The chloroform may also be filtered direct into the dish as fast as it evaporates. If the residue is bitter and can be scraped together with a platinum spatula or a pocket-knife, test for morphine and narceine.[1] In testing for morphine, use Froehde's, Husemann's and Pellagri's test, as well as that given by formalin-sulphuric acid. Presence of morphine is not established unless all these morphine tests give positive results! If the quantity of residue from chloroform permits, test for morphine with ferric chloride solution. This test is very characteristic of morphine but requires more than traces for a satisfactory result. Finally make Straub's biological test for morphine.

Purification of Impure Morphine

When the chloroform residue is too impure, especially if red or brown, it should be purified. Dissolve it in hot amyl alcohol and shake the solution thoroughly with several portions of hot water containing a few drops of dilute sulphuric acid. Acid dissolves morphine, whereas amyl alcohol retains most of the coloring matter. Add ammonium hydroxide solution, or sodium bicarbonate, to the acid solution until alkaline and extract several times with hot chloroform. Morphine obtained by evaporation of chloroform should be nearly pure.

Extraction of Free Morphine with Ether-Alcohol Mixture

To extract free morphine, Jörgensen[2] recommends ether containing alcohol, because it removes less extractive matter, the source of impurity of extracts from cadaveric material, than do the usual morphine solvents, such as amyl alcohol, chloroform or acetic ether. Slow evaporation of the clear ether extract gives as a rule entirely colorless, prismatic crystals of morphine that respond to the tests for this alkaloid in a manner admitting of no doubt. To prevent alcohol as much as possible from passing from ether into water, only 1–1.5 per cent. of alcohol is added to the ether, since too much alcohol in water will retard or entirely prevent passage of morphine into ether. Since ether containing alcohol is far inferior to the usual solvents of morphine, the number of extractions of the ammoniacal solution should be increased. At least ten such extractions are required. If the aqueous solution is quite impure, first remove morphine by extraction with hot amyl alcohol and then take the alkaloid from the latter by extraction with water containing hydrochloric acid. Add ammonia in excess to this acid solution and extract morphine with ether containing 1–1.5 per cent. of alcohol.

[1] Antipyrine, colchicin and caffeine may also be in this residue.

[2] G. Jörgensen: Detection of Morphine in Animal Organs. Zeitschr. f analyt. Chem. 49 (1910), 484.

According to the original instructions of the Stas-Otto method, free morphine is extracted not with hot chloroform but with amyl alcohol. Aside from the fact that it is unpleasant to work with this alcohol on account of its disagreeable odor, amyl alcohol is decidedly toxic. As a result of inhaling even small quantities of its vapor, sensitive persons may have nausea and severe pains in the head. In the experience of the author, morphine obtained from extracts with commercial amyl alcohol is not as pure and free from color as from chloroform extracts. For this reason years ago the author recommended warm chloroform for the extraction of morphine. In following the Stas-Otto method, Nagelvoort[1] recommends isobutyl instead of commercial amyl alcohol, since it is as good a solvent of morphine as the latter and is without its unpleasant odor.

MORPHINE

Opium contains several alkaloids combined almost exclusively with sulphuric and meconic acid. Of these alkaloids morphine is always present in largest quantity, that is, to the extent of 10 per cent. and more. In addition to its occurrence in the juice of the unripe capsules of the colored flowers of different varieties of Papaver somniferum, morphine is also found in smaller quantity together with other opium bases in all parts of these plants, such as leaves, stems and seeds, but only preceding maturity. As the ripening process advances, the juice in the plant diminishes and also the quantity of morphine, so that ripe poppy-heads contain at most only traces of the alkaloid.

Properties.—Morphine, $C_{17}H_{19}NO_3.H_2O$, crystallizes from dilute alcohol with 1 molecule of water of crystallization. It forms colorless, transparent, lustrous prisms that are only slightly soluble in water (1:5000 at 15° and 1:500 at 100°). These solutions are very bitter and have an alkaline reaction. Crystalline morphine is insoluble in ether and benzene. The amorphous alkaloid is soluble in amyl alcohol, isobutyl alcohol, hot chloroform and acetic ether. Ammonia, caustic alkalies, alkaline carbonates and bicarbonates precipitate the free base from solutions of morphine salts but, since morphine is a phenol, excess of caustic alkali holds it in solution.

Constitution.—Morphine as a monacid base combines with one equivalent of acid, forming as a rule salts that crystallize well. Their solutions have a neutral reaction and a very bitter taste. Morphine hydrochloride (Morphinum hydrochloricum), $C_{17}H_{19}NO_3.HCl.3H_2O$, the most important salt of this alkaloid, is extensively used in medicine. The tertiary character of morphine appears from the fact that it combines with methyl iodide in sealed tube at 100°, forming morphine-methyl iodide, $C_{17}H_{19}NO_3.CH_3I$, crystallizing in colorless lustrous needles that yield upon treatment with moist silver oxide the slightly stable, crystalline ammonium base, morphine-methyl hydroxide, $C_{17}H_{19}NO_3.CH_3.OH$.-

[1] J. B. Nagelvoort: Detection of Alkaloids in Toxicological-chemical Examinations. Nederl. Tijdschr. Pharm. 10 (1898), 616.

$5H_2O$. Fusion of morphine with caustic potash, or heating with soda lime, gives methyl amine, showing that a methyl-group is attached to nitrogen, that is, that this alkaloid contains the $:N.CH_3$ group. The three oxygen atoms possess different functions. Two form hydroxyl-groups, for with acetyl chloride morphine forms diacetyl-morphine, $C_{17}H_{17}NO(O.CO.CH_3)_2$, the hydrochloride of which is used in medicine under the name "Heroine:"

$$C_{17}H_{17}NO(OH)_2 + 2CH_3.COCl = 2HCl + C_{17}H_{17}NO(O.CO.CH_3)_2.$$

Similarly, benzoyl chloride gives dibenzoyl-morphine, $C_{17}H_{17}NO(O.CO.C_6H_5)_2$, crystallizing in needles. One hydroxyl-group must be phenolic in character, for morphine exhibits the properties of a monacid phenol. It is soluble in solutions of caustic alkalies but not in ammonia and for that reason is precipitated from these alkaline solutions by ammonium salts:

(α) $C_{17}H_{17}NO(OH)(OH).HCl + NaOH = NaCl + H_2O + C_{17}H_{17}NO(OH)(OH)$

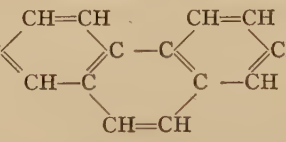
(β) $C_{17}H_{17}NO(OH)(OH) + NaOH = H_2O + C_{17}H_{17}NO(OH)(ONa)$

Sodium morphinate

(γ) $C_{17}H_{17}NO(OH)(ONa) + (H_4N)Cl = NaCl + NH_3 + C_{17}H_{17}NO(OH)_2.$

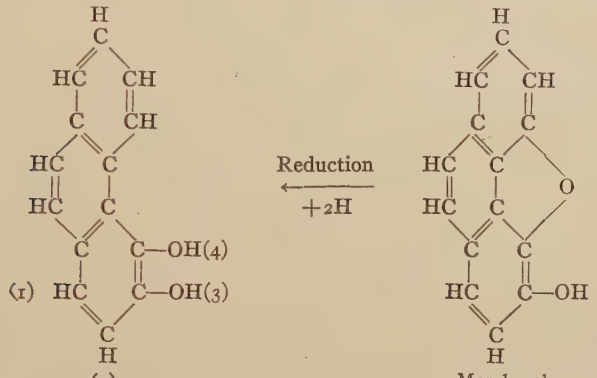
The phenolic character of morphine is shown by the fact that its neutral salts give a characteristic coloration with ferric chloride and that diazonium salts couple with morphine in alkaline solution forming azo-dyestuffs. The second oxygen atom of morphine is alcoholic in character; and the third is in ether-like union with two carbon atoms, a so-called bridge-oxygen atom.

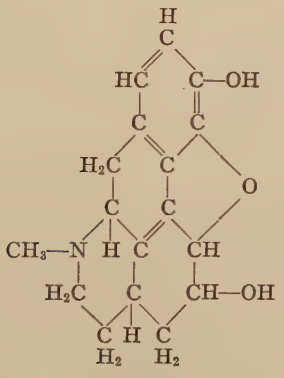
Of the 17 carbon atoms in morphine, 14 belong without question to a phenanthrene nucleus, for when morphine is heated with ten times the quantity of zinc dust, it gives off considerable ammonia and methyl amine, and a thick, brown liquid distils over consisting principally of phenanthrene.[1] More than that, well-defined phenanthrene derivatives have been obtained from derivatives of morphine by various reactions. If morphine-methyl iodide (see above) is boiled for several hours with acetic anhydride, the product together with other substances is diacetyl-dioxyphenanthrene, $C_{14}H_8(O.CO.CH_3)_2$, which gives morphol, $C_{14}H_8(OH)_2$, upon treatment with alcoholic ammonia. This compound has been prepared synthetically by Pschorr[2] and is identical with 3, 4-dioxyphenanthrene. A second derivative of phenanthrene is obtained from methyl-codeine methyl iodide, or from the corresponding ammonium base, which undergoes cleavage during dry distillation into trimethyl-amine, ethylene and methyl-morphenol. The latter heated with hydriodic acid is converted into morphenol,

[1] Phenanthrene, $C_{14}H_{10}$,  , occurs in coal-tar together with its isomer anthracene. It forms colorless crystals melting at 99° and boiling at 340°. It is readily soluble in ether or benzene and with difficulty in alcohol. Phenanthrene solutions exhibit bluish fluorescence.

[2] R. Pschorr: Constitution of Apomorphine. A Contribution to the Problem of the Constitution of Morphine. Ber. d. Deutsch. chem. Ges. 40 (1907), 1984.

$C_{14}H_7O(OH)$, which easily gives phenanthrene upon distillation with zinc dust and morphol upon reduction with sodium in absolute alcohol solution. Morphenol contains two hydrogen atoms less than morphol. The constitution of morphol has been definitely established by synthesis (Pschorr) and that of morphenol is probably as represented below. At the same time the formula of morphine proposed in 1907 by R. Pschorr is included:





Morphine is easily oxidized. This may be brought about in alkaline solution by atmospheric oxygen. Potassium permanganate, potassium ferricyanide, ammoniacal copper solution, and ammoniacal silver nitrate solution may also be used. As a result non-toxic oxy-dimorphine, also called pseudo-morphine which is soluble in caustic alkali, is formed:

$$2C_{17}H_{19}NO_3 + O = H_2O + C_{34}H_{36}N_2O_6.$$

The strong reducing action of morphine is due to the ease with which it undergoes oxidation.

Physiological Action.—Morphine is easily absorbed by the mucous membrane of the stomach, rectum, respiratory tract and open wounds. The action of

morphine is more rapid and stronger following hypodermic injection than when administered by way of the stomach. The entirely different reaction toward morphine of various animals is remarkable. Cold-blooded vertebrates are very slightly sensitive to morphine. To produce effects in a frog, more of the alkaloid must be injected than is required by man. Birds (pigeons and hens) can also stand surprisingly large doses of morphine. According to Guinard, the average toxic dose of morphine per kilogram of body-weight is 7 mg. for a horse, 15 mg. for a beef-creature, 40 mg. for a cat, 65 mg. for a dog, 200 mg. for a hog, 320 mg. for a rabbit, and 400 mg. for a goat. Considerable qualitative differences in the action of morphine have also been observed, since the brain of one group is primarily stimulated and of another group primarily paralyzed. At a second stage in the case of the second group increase in reflex-irritability of the spinal cord appears. In response to morphine, man is in the second group. Since his brain is exceedingly sensitive to poisons causing paralysis, the most pronounced action observed is that of a sedative and hypnotic (Kobert). In Europeans the stage of brain-stimulation seldom is noted, whereas it is said to occur more frequently in Malays and other inferior races. The stage of stimulation of the spinal cord in human adults is rarely observed, for long before that they die from paralysis of respiration. Morphine exerts a marked influence upon regulation of the temperature of the human body. If the environment is cold, the temperature drops below normal; if warm, fever appears. Under the influence of morphine, consumption of oxygen and escape of carbon dioxide are considerably diminished. During severe intoxication from morphine, respiration is slow and superficial.

Symptoms of morphine poisoning are soon in evidence, rarely later than 1–2 hours, and manifest themselves by: great somnolence, insensibility, deepest cyanosis, coma becoming deeper and deeper, pulse imperceptible and very slow, respiration rattling or entirely imperceptible, and body-temperature far below normal. Additional symptoms observed are first very brief dilatation of the pupils, then extreme contraction, and before death recurrence of dilatation. Death usually ensues in 6–8 hours from pulmonary oedema and cessation of respiration. In morphine poisoning terminating favorably, coma gradually gives place to quiet sleep often lasting 24–36 hours. The patient after awaking usually has headache and frequently emesis. In many cases constipation and digestive disturbances are very persistent. For an adult the smallest lethal dose of morphine is 0.2 gram and the average 0.4 gram. On the other hand, opiophagists and morphinists frequently tolerate several grams of morphine daily, since the organisms of such individuals can dispose of quite large quantities of morphine. There is habituation to the poison!

Elimination.—According to Marquis,[1] morphine disappears very rapidly from the blood, since it is held by certain organs, such as the brain. A portion of the morphine absorbed is conjugated with glycuronic or sulphuric acid (Baumann), another portion is oxidized, and the balance of the alkaloid is eliminated unchanged. After medicinal doses only little morphine, or none at all, appears in the urine. The urine of morphinists, however, is different. In such urines

[1] E. Marquis: Continuance of Morphine in the Animal Organism. Investigations of the Dorpat Institute edited by Kobert 14 (1896).

the author has repeatedly found quite considerable quantities of this alkaloid by the colorimetric method. In man and dogs a not immaterial part of the morphine taken is again eliminated by the glands of the gastro-intestinal tract and also when the alkaloid is injected subcutaneously.

According to Cloetta,[1] morphine in acute poisonings is carried on in the blood-plasma but completely disappears, 20 minutes at least after injection. Decomposition of morphine in the circulation is said not to occur. Lipoids of the brain abstract morphine from the plasma and there it is rather firmly held. One consequence of this is serious disturbance of the function of brain cells, but on the other hand the morphine molecule also undergoes decomposition. That part of the morphine not held by the brain is decomposed elsewhere in the body or eliminated. The ability of the organism to destroy morphine in acute poisoning differs with individuals. Oxidative processes play an important part in the decomposition of morphine. According to Cloetta, ferment action is excluded. In habituation the power of lipoids of the brain to combine with morphine increases, but at the same time increase in decomposition of the alkaloid takes place.

Contrary to the statements of Cloetta, Rübsamen[2] was unable to detect decomposition of morphine by the brain substance of normal animals outside the body. During acute morphine poisoning, vomitus, the stomach and contents, intestines, faeces, and especially the urine should be examined. Usually morphine can also be easily detected in the saliva. According to Marquis, the parts of the cadaver best suited for detection of morphine are liver and kidneys.

Stability of Morphine in Cadaveric Putrefaction.—According to Autenrieth,[3] morphine is only slightly decomposed, even during putrefaction lasting for a long time. In the stomach and contents of an adult, who took 25 grams of tincture of opium and died 4 hours later, he found 0.028 gram of nearly pure morphine. The urine was also found to contain morphine. After 18 months of continuous putrefaction, he found in what was left of the cadaveric material 0.02 gram of morphine, referred to the original quantity of material. Doepmann[4] after 11 months detected unchanged morphine in putrid meat to which it had been added. He extracted the meat with water containing acetic acid, evaporated the filtrate to small volume, precipitated with alcohol, and distilled the alcohol from the filtered liquid. The solution of the residue in water was filtered and precipitated with basic lead acetate. The filtrate from this precipitate was freed from lead by hydrogen sulphide. After removal of lead sulphide, the filtrate containing acetic acid was evaporated to about 100 cc. made alkaline with ammonia, and repeatedly extracted with hot chloroform. In the residue from chloroform Doepmann detected morphine positively by Husemann's and

[1] M. Cloetta: Behavior of Morphine in the Organism and Causes of Habituation to this Alkaloid. Arch. f. exper. Path. u. Pharm. 50 (1903), 453.

[2] W. Rübsamen: Experimental Studies of Habituation to Morphine. Arch. f. exper. Path. u Pharm. 59 (1908), 227.

[3] W. Autenrieth: Behavior of Morphine and Strychnine in Cadaveric Putrefaction. Ber. d. Deutsch. pharmaz. Ges. 11 (1901), 494.

[4] F. Doepmann: Experiments on Stability of Morphine during Putrefaction. Chem.-Ztg. 39 (1915), 69.

Pellagri's tests, by its behavior with Froehde's and Marquis' reagents, and also by Straub's biological test.

With regard to the stability of morphine during cadaveric putrefaction, Grutterink and van Rijn[1] have shown that they could detect this alkaloid with certainty after it had been in a cadaver for 2.5 years.

In an examination of the products of bacterial metabolism, human organs, consisting of liver, spleen, kidneys, heart, pancreas, intestines, etc., were subjected both to aerobic and anaerobic putrefaction, but no product was obtained giving tests that could be mistaken for those of morphine. Moreover these bacterial degradation-products do not interfere in any way with the detection of morphine. This alkaloid was added to mixtures undergoing putrefaction and could be easily detected by tests admitting of no doubt.[2]

Detection of Morphine

Evaporation of the chloroform, or alcohol-chloroform extract, leaves morphine as an amorphous, varnish-like residue, that only rarely becomes crystalline and has a very bitter taste. It should be carefully scraped together with a platinum or nickel spatula, or the clean blade of a pocket-knife, to get an idea of the approximate amount of substance available for tests. If the quantity is small, only specific tests for morphine should be made.

General alkaloidal reagents especially sensitive to solutions of morphine salts are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, gold chloride and phosphomolybdic acid. Platinic chloride produces an orange-yellow, granular precipitate only in concentrated solutions and then not until some time has elapsed.

Special Tests for Morphine[3]

1. **Nitric Acid Test.**—Concentrated nitric acid dissolves morphine with blood-red color changing at once to yellow. Addition of stannous chloride, or ammonium sulphide, fails to produce a violet color in this yellow solution (distinction from brucine).

2. **Husemann's Test.**—Concentrated sulphuric acid dissolves morphine without color. Heat this solution at once in a watch-glass for about 30 minutes upon a water-bath, or for a short time over a very small flame, until copious white fumes appear. A reddish

[1] A. Grutterink and W. van Rijn: Morphine in Parts of Cadavers. Pharm. Weekblad 52 (1915), 423.

[2] J. Rosenbloom and S. R. Mills: Non-conformity of Ptomaines with Certain Morphine Reactions. Journ. of Bio. Chem. 16 (1914), 327.

[3] Carry out these tests with free morphine base obtained by precipitation by ammonia from the aqueous solution of hydrochloride.

or more of a brownish color is produced. Cool this solution and add a drop of concentrated nitric acid. A fugitive red-violet color appears, almost instantly changing to blood-red and yellow-red and finally fading out entirely. Instead of warming the solution of morphine in concentrated sulphuric acid, allow it to stand in a desiccator at ordinary temperature for about 24 hours and then add a drop of concentrated nitric acid. A small crystal of potassium nitrate or chlorate may be substituted for nitric acid.

Frequently impure morphine is obtained from the chloroform extract of a solution prepared from cadaveric material. Such a residue gives a more or less highly colored solution with sulphuric acid. Heat usually intensifies this color. But even under these conditions it is possible to detect the red color caused by nitric acid or potassium nitrate.

3. **Pellagri's Test.**—Proceed with this test as described under codeine (see page 200). Avoid excess of alcoholic iodine solution, otherwise the latter may mask the green color.

Husemann's and Pellagri's tests depend upon conversion of morphine by concentrated sulphuric acid into apomorphine and detection of this base.

4. **Froehde's Test.**—This reagent dissolves morphine with a fine violet color, passing through blue into dirty green and finally into faint red. These colors vanish upon addition of water.

5. **Marquis' Test.**—Concentrated sulphuric acid containing formaldehyde dissolves morphine with a purple-red color, gradually passing into violet and finally into pure blue. This blue solution, kept in a test-tube and only slightly exposed to air, retains its color for some time.

Codeine and apomorphine give the same violet color. Narcotine also gives violet solutions but they become olive-green and finally yellow. Oxy-dimorphine gives a green color.

6. **Ferric Chloride Test.**—A few drops of neutral ferric chloride solution produce a blue color in a neutral solution of a morphine salt. This color gradually fades. It is due to the oxidizing action of ferric chloride upon morphine.

In making this test dissolve the chloroform residue in very dilute hydrochloric acid. Evaporate this solution to dryness upon the water-bath. Dissolve the residue in pure water and add a drop of ferric chloride solution.

7. **Iodic Acid Test.**—Shake a dilute sulphuric acid solution of morphine with a few drops of aqueous solution of pure iodic acid

(HIO_3), or potassium iodate (KIO_3) free from potassium iodide, and a little chloroform. The latter will have a violet color.

8. Brouardel-Boutmy Test.—An aqueous solution of a morphine salt is colored deep blue by a few drops of a very dilute mixture of potassium ferricyanide and ferric chloride solution. Considerable morphine will produce a blue precipitate of Prussian blue.

Morphine is oxidized by potassium ferricyanide to oxy-dimorphine:
$2C_{17}H_{19}NO_3 + 2KOH + 2K_3Fe(CN)_6 = 2H_2O + (C_{17}H_{18}NO_3)_2 + 2K_4Fe(CN)_6$.
Potassium ferrocyanide formed at the same time reacts with ferric chloride producing Prussian blue.

The basis of the tests with iodic acid and potassium ferricyanide (Tests 7 and 8) is the reducing action of morphine. Therefore other reducing substances, such as hydrastine, also give these two tests. There are also many ptomaines that exert a strong reducing action and consequently give the Brouardel-Boutmy test.

9. Nitrous Acid Test.—Add a small particle of sodium nitrite to a very dilute morphine salt solution, then a dilute acid and render alkaline with concentrated potassium hydroxide solution before all the gas has escaped. According to the concentration of the morphine solution, the mixture will be pale rose, orange-red to deep ruby-red. Acids discharge and alkalies restore the color.

In this test nitroso-morphine, $C_{17}H_{18}N_2O_4.H_2O$, is probably formed. Wieland[1] has obtained this compound in pure condition by passing nitrous acid (prepared from arsenic trioxide and concentrated nitric acid) into an aqueous solution of morphine hydrochloride cooled to about $-3°$. The sodium compound from 90 per cent. alcohol forms garnet-red needles. Free nitroso-morphine, obtained from this alkali salt with acetic acid, crystallizes in dark orange-red needles melting at 225°.

10. Donath's Test.—Triturate at least 1 mg. of morphine in a small porcelain dish with 8 drops of concentrated sulphuric acid. Add a small particle of potassium arsenate, triturate again, and warm over a small flame until fumes of sulphuric acid appear. A blue-violet color, passing into dark brown-red after further warming, will appear. Careful dilution with water develops a reddish color that becomes green upon addition of more water. If the latter is shaken in a test-tube with a little chloroform, this solvent will acquire a fine violet color.

[1] H. Wieland and P. Kappelmeier: Researches upon Morphine I. Annalen d. Chemie 382 (1911), 306.

11. Lloyd's Test.—Triturate morphine (5–8 parts) with hydrastine (1 part) and a few drops of concentrated sulphuric acid with a glass rod for 5–10 minutes. In the center the mixture is red-violet and blue-violet in the thinner marginal region. This color is similar to that produced by potassium dichromate in a sulphuric acid solution of strychnine. Apomorphine also gives Lloyd's test. Aconitine, atropine, berberine, brucine, and other alkaloids also give colors with hydrastine and sulphuric acid. Wangerin[1] considers these reactions characteristic only when 5–10 mg. of morphine and 2–10 mg. of hydrastine are present.

12. Schneider-Weppen Test.—Add to a colorless concentrated sulphuric acid solution of morphine a small particle of cane-sugar, or add morphine mixed with about 4 times the quantity of finely powdered sugar to concentrated sulphuric acid. The mixture has a red color that becomes more intense upon addition of a drop of bromine water.

13. Flückiger's Test.—Sprinkle upon a solution of morphine in concentrated sulphuric acid a little subnitrate of bismuth. A black-brown color will appear.

14. Azo-dyestuff Test.—Morphine is a phenol and will couple with diazonium salts forming azo-dyestuffs.

Dissolve morphine in dilute acetic acid. Cool well and add a cold diazonium salt solution prepared from aniline and containing sufficient sodium acetate to discharge mineral acid. Then drop in ice-cold sodium carbonate solution until the reaction is alkaline. A cinnabar-red body appears, soon changing into stable phenyl-azo-morphine, $C_{22}H_{23}N_3O_3$, crystallizing from hot alcohol in orange-yellow needles (H. Wieland).

By diazotized sulphanilic acid, morphine may be determined quantitatively by the colorimetric method. Details of this method are given in Chapter V of this book (see page 563).

15. Biological Test of Straub and Hermann.[2]—If an aqueous solution of morphine hydrochloride is injected under the skin of the back of a white mouse, after a few minutes the back takes on lordotic curvature. Slight spastic paresis = incomplete paralysis appears in the hind legs, so that they are held more strongly than usual in an extended position and it appears difficult for the animal to move. The tail begins to rise in an S-shaped curve until it finally lies over the

[1] A. Wangerin: Contribution to Lloyd's Reaction for Morphine. Pharm. Ztg. 48 (1903), 57.

[2] O. Hermann: A Biological Method of Detecting Morphine. Biochem. Zeitschr. 39 (1912), 216.

back of the animal so that the tip is over the ears. The entire behavior of the mouse gives the impression of increased restlessness and reflex-irritability, so that it jumps up terrified at faint noises, especially from acoustic stimulation of high sounds. The position of the tail is most striking and regular and is increased by running about. It is advisable to place the animals under a bell-jar upon a wooden plate and alarm them by striking the jar with a glass rod. Use white mice weighing 16–20 grams. After injection of 5 mg. of morphine the reaction lasts about 20 hours, falling rather uniformly, according to the dose, to 45 minutes after 0.005 mg. The duration of the maximum of the reaction amounts to 4.5 hours for 5 mg., falling to 30–45 minutes for 0.01 mg. of morphine. From a forensic point of view this reaction by itself is not sufficiently convincing. Yet it may be used as a preliminary test, or to confirm what chemical tests have shown to be probable. Its practical application is due chiefly to the fact that crude morphine obtained by the Stas-Otto method may be used direct for the biological test, thus avoiding purification of the alkaloid which is usually attended with considerable loss of material.

In Chapter V of this book detection of morphine in animal organs according to the procedures of Cloetta and Marquis is explained in full.

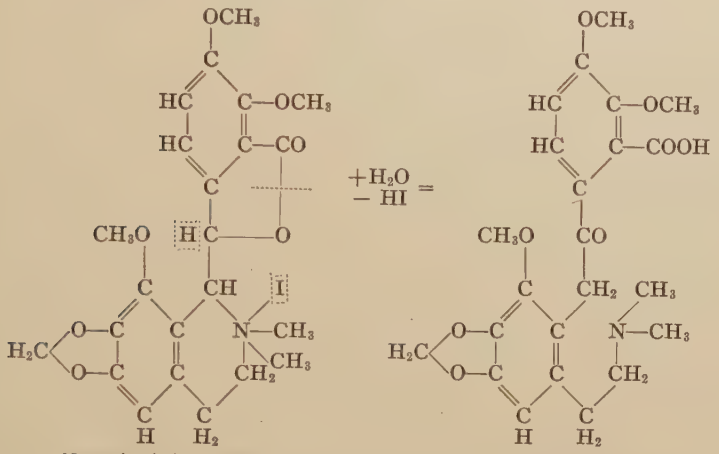
NARCEINE

Narceine is found in opium to the extent of 0.1–0.4 per cent. In chemical nature it is closely related to narcotine from which it may be prepared (see below).

Properties and Constitution.—Narceine, $C_{23}H_{27}NO_3.3H_2O$, crystallizes from water or alcohol in long, white prisms frequently in clusters, melting when air-dried at 165°. This alkaloid has a faintly bitter taste. It is slightly soluble in cold but freely soluble in hot water. When a hot saturated aqueous solution of narceine is cooled, it solidifies to a crystalline mass. Narceine is insoluble in ether, benzene or petroleum ether and only slightly soluble in cold alcohol, amyl alcohol and chloroform, though freely soluble in these solvents at boiling temperature. Solutions of narceine react neutral to litmus and are optically inactive. Narceine is more abundantly soluble in aqueous ammonia, or caustic alkalies, than in pure water.

Narceine is a tertiary base in which two methyl-groups are attached to nitrogen, for it combines with alkyl iodides to form ammonium iodides. With methyl iodide it gives narceine iodo-methylate, $C_{23}H_{27}NO_3.CH_3I$. By Zeisel's method it may be shown that the molecule also contains three methoxyl-groups. Since narceine is soluble in caustic alkalies and ammonia and also forms esters with alcohols, it must contain a carboxyl-group. This alkaloid must also contain a carbonyl-group (CO), since it forms a hydrazone with phenyl-hydrazine. The narceine molecule contains neither an alcoholic nor a phenolic hydroxyl-group, since it forms no acetyl-derivative when heated with acetic anhydride. Upon the basis of these facts the narceine formula may be resolved into: $C_{16}H_{11}NO(CH_3)_2(OCH)_3(CO)(COOH)$. From a chemical point of view narceine is closely related to narcotine. By heating narcotine iodo-methylate with sodium hydroxide solution, Roser converted it into a base called pseudo-

narceine. Freund has recently shown that Roser's pseudo-narceine is identical with the opium alkaloid narceine and explains the conversion of narcotine into narceine by assuming that the iodo-methylate loses a molecule of hydriodic acid and takes up a molecule of water:



Narcotine iodo-methylate Narceine

All reactions and transformations of narceine may be easily explained upon the basis of this structural formula.

Physiological Action.—The action of narceine is similar to that of morphine but milder. It is probably only slowly absorbed, for it can be found for quite a long time in the stomach and intestines. Elimination takes place principally in the urine and bile. Dragendorff has also found it in the blood and in organs rich in blood. In doses of 0.01–0.02 gram narceine is used as a sedative and antispasmodic, and as a hypnotic in doses of 0.03–0.1 gram. This alkaloid is used in the form of the free base and as the hydrochloride and meconate. The combination sodium narceinate-sodium salicylate ($C_{23}H_{26}NO_3Na + 3C_6H_4(OH)$-$COONa$) is used in medicine under the name "Antispasmine." Secondary effects, observed after doses of 0.03–0.1 gram of narceine, are: dryness in the mouth, emesis, slowing of heart-action, dysuria, itching of the skin, and increased secretion of sweat (Lewin).

Detection of Narceine

Narceine possesses at the same time the characteristics of a weak base and of a weak acid. Consequently it acts like a base with the stronger acids and like an acid with strong bases, forming salts that are, however, in aqueous solution extensively hydrolyzed. This amphoteric character of narceine explains why it cannot be extracted by ether, benzene or petroleum ether from a solution that is either acid or alkaline from sodium hydroxide. Extraction takes place better, if, as in the case of morphine, the solution is rendered alkaline with ammonia or sodium bicarbonate and shaken with hot chloroform or amyl alcohol. According to Kippenberger, chloroform containing 10 per cent. of alcohol even in the cold

completely extracts narceine from a solution alkaline from sodium bicarbonate. The general alkaloidal reagents that are more sensitive to narceine are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide and phospho-molybdic acid.

Special Reactions of Narceine

1. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves narceine with a yellow to yellow-brown color, changing in the cold within a few hours, and immediately upon warming, to blood-red.

2. **Dilute Sulphuric Acid Test.**—If narceine is warmed in a small porcelain dish upon the water-bath with dilute sulphuric acid, at a certain concentration the acid takes on a fine violet color, changing to cherry-red upon longer heating. If a trace of nitric acid is added to this cherry-red solution when cold, blue-violet streaks will appear.

3. **Froehde's Test.**—This reagent dissolves narceine with a brown-green color, gradually passing into green and finally into red. Gentle heat favors this color-change. If somewhat larger quantities of narceine are warmed until the color is red and the solution is allowed to cool, a fine corn-flower blue will extend out from the margin.

4. **Erdmann's Test.**—This reagent, as well as concentrated nitric acid, dissolves narceine with yellow color.

5. **Vogel's Test.**—Pour a few drops of chlorine water upon narceine and add, while stirring, a few drops of ammonia. A deep red color at once appears, persisting in presence of excess of ammonia and upon warming.

6. **Iodine Test.**—Aqueous iodine solution (iodine water), or iodine vapor, colors solid narceine blue. Morphine strongly influences the delicacy of this reaction.

7. **Potassium Zinc Iodide Test.**—With 0.5 mg. of narceine, even in dilution of 1:1000, this reagent produces a precipitate of white, filiform crystals that turn blue after a time. This blue color appears immediately, if a trace of iodine solution is added to the reagent.

8. **Resorcinol-Sulphuric Acid Test.**—Rub together upon a watch-glass 0.01–0.02 gram of resorcinol with 10 drops of concentrated sulphuric acid, add a trace of narceine (0.002–0.005 gram), and while stirring warm the intensely yellow solution upon a boiling water-bath. A fine crimson-red to cherry-red color will appear. As the solution cools, this color, beginning at the margin, gradually passes through blood-red and after several hours into orange-yellow.

9. **Tannin-Sulphuric Acid Test.**—Heat upon a water-bath while stirring 0.002–0.01 gram of narceine with 0.01–0.02 gram of tannin and 10 drops of concentrated sulphuric acid. The solution at first yellow-brown immediately becomes pure green. If heat is applied for some time, the green color changes to blue-green and finally through a more or less blue tone to a dirty green.

Narcotine and hydrastine give a similar green color-reaction with tannin-sulphuric acid. In chemical structure these two alkaloids are related to narceine. Veratrine gives a red color, whereas most of the other alkaloids give more or less brown colors that are not characteristic. (Wangerin.[1])

[1] A. Wangerin: Color Reactions of Narceine. Pharmaz. 47 (1902), 916.

Behavior of More Important Alkaloids toward Concentrated Nitric Acid, Concentrated Sulphuric Acid, and Reagents[1] of Froehde, Marquis, Mandelin, Mecke, Rosenthaler and Schaer

The more important alkaloids give with the above reagents colors that are more or less characteristic.

Pure Concentrated Nitric Acid (Sp. Gr. 1.40 = 61 per cent.) dissolves:

Berberine: red-brown,
Brucine: blood-red, immediately yellow,
Codeine: yellow to orange,
Colchicin: violet, then dirty greenish brown,
Morphine: red-yellow,
Narceine: fugitive yellow,
Narcotine: lemon-yellow,
Papaverine: yellow, then orange,
Physostigmine: yellow,
Strychnine: yellow,
Thebaine: yellow
Veratrine: faintly yellowish.

The following are soluble without color: aconitine, atropine, quinine, caffeine, cocaine, coniine, nicotine, theobromine and cytisine.

Pure Concentrated Sulphuric Acid dissolves:

Berberine: olive-green, then yellow,
Digitalin: orange-yellow and red,
Emetine: pale brownish,
Colchicin: yellow
Curarine: red,
Narcotine: pale yellow, gradually yellow-red,
Narceine: yellow, soon brown-yellow,
Papaverine: violet-blue,
Physostigmine: yellow, soon green.
Picrotoxin: orange-yellow,
Solanine: reddish yellow,
Thebaine: blood-red, later yellow-red,
Veratrine: yellow with green fluorescence, then orange and cherry-red.

If not warmed, the following are soluble without color: atropine, brucine, quinine, hydrastine, codeine, caffeine, cocaine, morphine, nicotine, pilocarpine, strychnine, theobromine and cytisine.

Froehde's Reagent, or Molybdic-sulphuric Acid[2] dissolves:

Berberine: brown-green,
Brucine: raspberry-red, brownish yellow,
Codeine: greenish, upon warming blue,
Colchicin: yellow,
Curarine: violet,
Morphine: violet-red, then green,
Narceine: yellow-brown,
Narcotine: blue-green, green, reddish yellow,
Papaverine: violet-blue, then yellow,
Solanine: yellow-red, red-brown, yellow,
Thebaine: red, red-yellow,
Veratrine: yellow, orange, cherry-red.

The following give no color with Froehde's reagent: atropine, quinine, cocaine, caffeine, nicotine, strychnine, theobromine, cinchonine and cytisine.

[1] For the preparations of these reagents, see pages 641 to 643.

[2] Froehde's reagent does not keep long. Therefore it should be freshly prepared, or what is on hand should be tested with morphine to determine its fitness for use.

Mandelin's Reagent, or Vanadic-sulphuric Acid dissolves:

Berberine: dirty green, later brown,
Brucine: red, yellow,
Emetine: brown,
Codeine: green, upon warming blue,
Colchicin: blue-green, green, brown,
Curarine: violet,
Morphine: red, then blue-violet,
Narcotine: cinnabar-red to crimson-red,
Narceine: violet, later red-yellow,
Papaverine: blue-green, then blue,
Solanine: orange-red, later violet,
Strychnine: blue-violet,
Thebaine: orange-red,
Veratrine: yellow, cherry-red.

The following give no color with Mandelin's reagent: atropine, quinine, cocaine, caffeine, coniine, nicotine, theobromine, cinchonine and cytisine.

Marquis'[1] Reagent, or Formalin-suphuric Acid dissolves:

Apomorphine: violet, rusty red, dark blue,
Dionine: pure blue,
Heroine: red, then blue-violet,
Codeine: reddish, blue-violet, violet-blue,
Morphine: peach-red, violet, blue-violet, pure blue,
Narcotine: violet, olive-green, yellow,
Papaverine: wine-red, yellow, dirty brown-red, deep orange,
Peronine: red-violet,
Veratrine: yellow-brown, upon warming reddish brown.

Mecke's[2] Reagent, or Selenious-sulphuric Acid dissolves:

	In the Cold	Upon Warming
Apomorphine:	dark blue-violet,	dark brown,
Brucine:	yellow-red,	lemon-yellow,
Codeine:	blue, quickly emerald-green, later olive-green,	steel-blue, then brown,
Colchicin:	lemon-yellow,	yellowish brown,
Morphine:	blue, blue-green to olive-green,	brown,
Narceine:	greenish yellow, then violet,	dark violet,
Narcotine:	greenish, steel-blue, then cherry-red,	cherry-red,
Papaverine:	greenish, dark steel-blue, then deep violet,	dark violet,
Physostigmine:	brownish yellow,	pale brown-red,
Thebaine:	deep orange, gradually fading,	dark brown,
Veratrine:	lemon-yellow, olive-green.	brownish violet.

[1] E. Marquis: Distribution of Morphine in the Animal Body. Wiener mediz. Presse 1896 No. 42 and Pharmaz. Zentralhalle 37 (1896), 844.

[2] A. Mecke: A New Reagent for Alkaloids. Zeitschr. f. öffentl. Chem. 5 (1899), 350; Zeitschr. f. analyt. Chem. 39(1900), 468. See "Preparation of Reagents" page 643.

Mecke's reagent therefore is preeminently a sensitive reagent for most opium alkaloids. A great advantage of this reagent is the fact that it gives characteristic colors when alkaloids are not in a state of perfect purity.

Rosenthaler's[1] Reagent or Arsenic-Sulphuric Acid

Procedure.—Dissolve a small particle of the substance to be tested in 2–3 cc. of this reagent and warm the solution in a boiling water-bath. Fine colors that are very stable appear, if certain alkaloids are present. Usually these reactions are quite sensitive, giving distinct tests with 0.05 mg. of morphine, 0.02 mg. of narcotine, 0.1 mg. of codeine, dionine or apomorphine. A notable fact is that the alkaloids, hydrastine and hydrastinine, related to narcotine, also bear a close resemblance to this alkaloid in their behavior toward sulphuric acid containing arsenic acid. Of the other alkaloids only berberine and brucine give colors deserving mention but they are less distinct and not as fine.

	Cold	Warm	Warm, and after Addition of Hydrochloric Acid
Codeine:	light blue	dark blue	purple-red
Morphine:	gradually greenish blue, then green	super-blue quickly emerald-green, later dark green	red-violet
Apomorphine:	super-yellow-green and green in blue	green	brown
Heroine:	yellow-brown with tinge of red	dark black-green	dark cherry-red
Dionine:	yellow	first blue, then green	purple-red, with a tinge of violet
Narcotine:	greenish yellow	cherry-red	yellow-red
Hydrastine:	yellow	cherry-red	yellow-red

Schaer's Reagent[2] or Perhydrol-Sulphuric Acid

Perhydrol, dissolved in concentrated sulphuric acid, gives fine color-reactions with various alkaloids. This reagent is prepared by carefully mixing 1 volume of 30 per cent. chemically pure "*Perhydrol*" with 10 volumes of pure concentrated sulphuric acid. This

[1] L. Rosenthaler and F. Türk: Sulphuric Acid containing Arsenic Acid as a Reagent for Alkaloids. Apoth.-Ztg. 19 (1904), 186.

[2] E. Schaer: Reactions of Alkaloids with Perhydrol. Archiv d. Pharmaz. 248 (1910), 458.

reagent should always be freshly prepared. Use for each test 1 cc. of the cooled reagent and 5–10 mg. of the alkaloid. Quinine, or a quinine salt, gives a lemon-yellow to canary-yellow color. Strychnine in perhydrol-sulphuric acid, to which a small quantity of colloidal platinum solution has been added, gives slowly, usually not for several hours, a faint purple-red color that is characterized by great stability. This reagent, after previous addition of a little platinum solution, gives with brucine more of an orange-red, without platinum a reddish yellow. Morphine, codeine, narcotine, narceine and papaverine give with this perhydrol-reagent alone, or after addition of a little platinum solution, orange-red to purple-red, partly dark brown-yellow colors that soon disappear. Therefore these reactions are of little use in identifying opium alkaloids. Berberine gives a dark cherry-red with the perhydrol-reagent, gradually passing into brown-red. Hydrastine produces an intense chocolate-brown color that is especially distinct and comes quickly, if a little platinum solution has first been added to the reagent. According to Wasicky,[1] perhydrol-sulphuric acid also gives fine color-reactions with atropine, hyoscyamine and scopolamine. If a few drops of this reagent are poured upon a small quantity of one of these bases, the particles of the alkaloid as they pass into solution, that is, first at the edges, give an intense leaf-green color. After a few minutes this color passes into more of an olive-green and finally takes on a discolored brown-green appearance. Homatropine also gives this test. It also appears with cocaine but comes somewhat later and the color is more of an emerald-green.

Silicic-tungstic Acid or Godeffroy's Alkaloidal Precipitant

In 1876 Godeffroy[2] called attention to the great sensitiveness of silicic-dodeca-tungstic acid toward alkaloids and among others found that atropine was precipitated in a dilution of 1:15,000, quinine 1:50,000 and cinchonine 1:200,000 by this acid. Bertrand[3] later subjected Godeffroy's reaction to a more critical examination.

Silicic-tungstic acid, $12WoO_3.SiO_2.2H_2O$, forms perfectly stable, well-defined salts. In 5 per cent. aqueous solution it may be used as such or as an alkali salt. The sensitiveness of the reaction exceeds nearly all the general tests for alkaloids

[1] R. Wasicky: A New and Very Sensitive Color-reaction of Atropine, Hyoscyamine and Scopolamine. Zeitschr. f. analyt. Chem. 54 (1915), 393.

[2] R. Godeffroy: Use of Silicic-Dodeca-Tungstic Acid as a Reagent for Alkaloids. Ber. d. Deutsch. chem. Ges. 9 (1876), 1792.

[3] G. Bertrand: Compt. rend. 128,742 and Bull. de la Soc. chim. de Paris (3 série) 21 (1903), 434.

thus far recommended. With cold sufficiently concentrated solutions of alkaloidal salts this reagent usually produces precipitates that are flocculent, gather into balls, are pulverulent, or even crystalline. They are white, faintly yellowish or reddish in color and can easily be filtered from the liquid. These precipitates are almost insoluble in cold water and but slightly soluble at boiling temperature. In composition they have the general formula:

$$12WoO_3.SiO_2.2H_2O.\text{Alkaloid} + nH_2O.$$

These silico-tungstates are insoluble even in concentrated acids and upon ignition give a residue consisting of $12WoO_3.SiO_2$.

Morphine silico-tungstate, $12WoO_3.SiO_2.2H_2O.4C_{17}H_{19}NO_3 + 9H_2O$, is amorphous, salmon-colored and contains, when dried at 120°, $2H_2O$.

Strychnine silico-tungstate, $12WoO_3.SiO_2.2H_2O.4C_{21}H_{22}N_2O_2 + 8H_2O$, is similar in appearance to the morphine salt and contains $1H_2O$ when dried at 120°.

If after precipitation liquid and precipitate are heated together, the latter loses a certain quantity of water and then forms a finely divided powder that settles rather slowly. In the case of various alkaloids the precipitate in this condition is more distinctly visible than when precipitated cold.

In establishing the limit of delicacy, Bertrand added for each 5 cc. of alkaloidal salt solution 1–2 drops of the silicic-tungstic acid reagent and the same quantity of 10 per cent. hydrochloric acid. He found the limit for coniine 1:8000, nicotine 1:20,000, narceine 1:30,000, codeine 1:40,000, atropine and caffeine 1:50,000, and cocaine 1:200,000. It made no difference with these alkaloids whether they were precipitated from hot or cold solution. The sensitiveness of the following bases was much greater, if they were first heated to boiling and then allowed to cool, and was found to be for aconitine 1:80,000, veratrine 1:130,000, brucine 1:150,000, strychnine and narcotine 1:200,000, and quinine and cinchonine 1:500,000.

Alkaloidal silico-tungstates are easily attacked by oxidizing agents. This fact makes it possible to use these precipitates for those characteristic color-reactions that depend upon processes of oxidation, for example, the test for strychnine with sulphuric acid and potassium dichromate.

Alkaloidal silico-tungstates are at once decomposed even in the cold by dilute aqueous solutions of caustic alkalies and also by ammonia. In this way the free alkaloidal bases can easily be separated from these precipitates. They may then either be filtered off, or better extracted by an appropriate solvent, such as ether or chloroform. In this procedure silicic-tungstic acid remains in the aqueous alkaline liquid as a salt.

CHAPTER III

METALLIC POISONS

Fresenius-v. Babo[1] Procedure for Destruction of Organic Matter

The residue left after removal of volatile poisons by steam-distillation may be used in this part of the analysis, as it must contain poisonous metals if any are present. A portion of the original material,[2] previously finely chopped and well-mixed with enough water to produce a fluid mass, may also be used. According to the quantity of material to be destroyed, add 10–15 cc. of pure concentrated hydrochloric acid and 1–2 grams of potassium chlorate. Set the flask upon a boiling water-bath in a good draught, heat and shake frequently so that chlorine comes into intimate contact with material to be destroyed. When the mixture is hot, add 0.3–0.5 gram of potassium chlorate every 2–3 minutes and shake the flask frequently. Continue in this manner until most of the organic matter is dissolved and the liquid in the flask is clear, or turbid, and has a wine-yellow color. Further addition of potassium chlorate and longer heating should produce no change. Fat and fatty acids in particular are extremely resistant to the action of chlorine.

When organic matter is completely destroyed, dilute with hot water, adding a few drops of dilute sulphuric acid to precipitate possible barium, shake and pour the cold liquid through a moistened filter. If the excess of free hydrochloric acid is not too large, the filtrate may be saturated direct with hydrogen sulphide. Otherwise, evaporate the solution in a porcelain dish upon the water-bath nearly to dryness to remove most of the free hydrochloric acid. This step

[1] R. Fresenius and L. v. Babo: A New Method dependable under all Conditions for Detection and Quantitative Estimation of Arsenic in Cases of Poisoning. Annalen d. Chem. u. Pharm. 49 (1844), 287.

[2] Cadaveric material should be divided as finely as possible, then brought to a thin mixture by stirring with 12.5 per cent. arsenic-free hydrochloric acid, and heated with frequent shaking with 1–2 grams of potassium chlorate as directed above. If the material is heated upon the water-bath in a porcelain dish, it should be stirred constantly.

frequently produces a dark color which a few crystals of potassium chlorate will discharge.

An alternative procedure is to remove part of the free hydrochloric acid from the filtrate, obtained after treatment with hydrochloric acid and potassium chlorate, by first evaporating to a smaller volume and then adding ammonia until alkaline. Finally add dilute nitric acid until the solution is faintly acid. Then saturate the liquid, which should not contain too much free acid, with arsenic-free hydrogen sulphide as directed upon page 246.

The insoluble residue upon the filter, obtained by heating the distillation-residue, or the original material, with hydrochloric acid and potassium chlorate, may contain silver chloride, barium sulphate and lead sulphate in addition to fat. Examine as directed under "Metallic Poisons IV" for silver, barium and lead (see page 262).

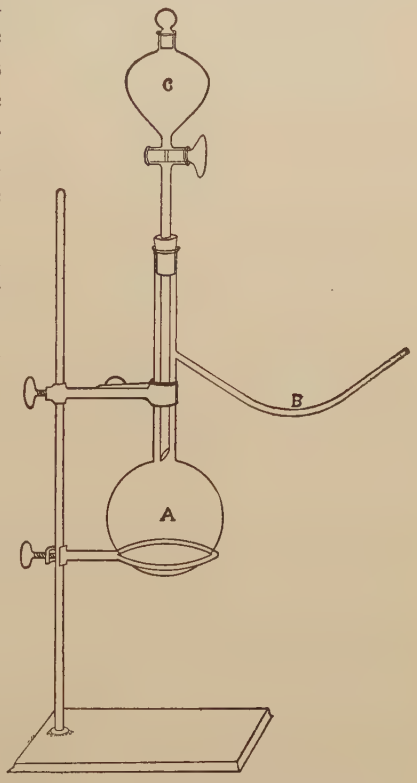


FIG. 17.—Thoms flask for destroying organic matter.

Thoms[1] destroys organic matter in the apparatus shown in Fig. 17. Oxidation is carried on in an ordinary fractioning flask (A) with the tubulus (B) bent upward. A separating funnel (C), held in the neck of the flask by a stopper, contains an aqueous solution of potassium chlorate (1:20) saturated at ordinary temperature. Organic matter, for example, finely divided organs, or other cadaveric material, is in the flask as a thin mixture with 12.5 per cent. hydrochloric acid. Add about 1 gram of solid potassium chlorate and warm the flask upon a boiling water-bath. When the mass in the

[1] H. Thoms: Introduction to the Practical Chemistry of Foods. Published by S. Hirzel, Leipsic, 64 illustrations and 163 pages.

flask is warm, allow the potassium chlorate solution to run in drop by drop and shake constantly. Care must be taken not to add too much of this solution at once, otherwise explosions may easily occur from formation of chlorine dioxide (ClO_2). In other respects the procedure is identical with that described above.

Notes.—Potassium chlorate and hydrochloric acid evolve chlorine (α and β), part of which acts upon the organic material destroying it, and part in presence of water forms oxygen and oxygen-acids of chlorine (γ and δ) which are strong oxidizing agents:

$$(\alpha)\quad KClO_3 + HCl = HClO_3 + KCl,$$
$$(\beta)\quad HClO_3 + 5HCl = 6Cl + 3H_2O,$$
$$(\gamma)\quad Cl_2 + H_2O = 2HCl + O,$$
$$(\delta)\quad Cl_2 + H_2O = HOCl + HCl.$$

Arsenious acid, present in the material, probably is not volatilized as arsenic trichloride ($AsCl_3$) by the procedure described but oxidized to non-volatile arsenic acid:

$$H_3AsO_3 + H_2O + Cl_2 = H_3AsO_4 + 2HCl.$$

Even after most thorough treatment of parts of the cadaver, or vegetable substances, with hydrochloric acid and potassium chlorate, a more or less abundant white precipitate, consisting in part at least of fat and free fatty acids (chlorinated fatty acids) and exceedingly resistant to the action of nascent chlorine, always remains. This treatment converts a portion of the organic matter into volatile compounds (chloranil?) which have a sharp odor and attack the mucous membranes. For this reason destruction of organic matter should take place in a hood with a good draught.

Treatment with hydrochloric acid and potassium chlorate, as described, converts metallic poisons into inorganic salts, usually chlorides and sulphates, which either remain in solution, or are precipitated as difficultly soluble silver chloride, barium sulphate, and in part also as lead sulphate. Protein substances, present in all animal and vegetable organisms and fluids, precipitate many heavy metals, such as mercury, silver, lead, copper, uranium and zinc, from solutions of their salts. These metals are then in the form of metallic albuminates, which usually dissolve in water with great difficulty and are very stable. As a rule these metallic protein compounds must receive further treatment before it is possible to detect the metal. Many organic acids, such as tartaric acid and carbohydrates, interfere more or less with the detection of heavy metals. In combination with these organic substances, heavy metals behave like copper in potassium cupro-cyanide, $K_6Cu_2(CN)_8$, which is precipitated by neither sodium hydroxide nor hydrogen sulphide, because in solution it is electrolytically dissociated, as shown in the equation, into the complex cupro-cyanide ion and not into copper ion:

$$K_6Cu_2(CN)_8 \rightleftarrows 6K^+ + Cu_2(CN)_8''''''.$$

If potassium cupro-cyanide is heated with hydrochloric acid and potassium chlorate, copper passes into solution as cupric chloride. The reagents mentioned above will then precipitate copper, for copper chloride is ionized as follows:

$$CuCl_2 \rightleftarrows Cu^{++} + 2Cl'.$$

Consequently the solution of cupric chloride will give the reactions of copper ion.

If metallic poisons are to be detected by ordinary ionic reactions, interfering organic substances, especially protein bodies, must be removed, that is, destroyed by some procedure whereby metals are converted into inorganic salts that undergo ionization.

Potassium chlorate acts best upon organic substances in strong hydrochloric acid solution. If the mass becomes too thick at any time during heating, it should be diluted with water or dilute hydrochloric acid. The contents of the flask should also be well-shaken during treatment with potassium chlorate, to prevent collection of a large quantity of this salt upon the bottom of the flask. Such an occurrence may cause an explosion due to formation of the exceedingly unstable chlorine dioxide (ClO_2):

$$(\alpha)\ KClO_3 + HCl = KCl + HClO_3,$$
$$(\beta)\ 3HClO_3 = HClO_4 + 2ClO_2 + H_2O.$$

In examining cadaveric material, the author employs 12.5 per cent. hydrochloric acid (sp. gr. 1.061) saturated with hydrogen sulphide and kept in a loosely stoppered bottle. This insures precipitation of the final traces of arsenic sometimes present even in the purest commercial acid.[1] When needed, this acid is filtered through ash-free paper to remove precipitated sulphur that may contain arsenic sulphide.

Cadaveric material is destroyed by the mixture of hydrochloric acid and potassium chlorate with relative rapidity and for the most part passes into solution. An experiment, in which 100 grams of stomach and duodenum, 20 grams of stomach-contents, 75 grams of kidney, and 200 grams of liver (395 grams in all) were treated as described, required about 1 hour for complete solution. The insoluble part, filtered, washed and dried upon a porous earthen plate, weighed 52 grams, and 32 grams when dried at 100°. It was an amorphous, yellowish white, fatty mass and almost entirely soluble in hot sodium hydroxide solution.

Vintilesco's[2] Modification of Fresenius-v. Babo Method of Destroying Organic Matter

After a critical discussion of the methods of Gautier, Denigès, Meillère, Kerbosch and Breteau, Vintilesco recommends the potassium chlorate method as best for destruction of organic substances in toxicological examinations, especially when carried out in the following manner:

Mix 200 grams of finely comminuted material in a porcelain dish with 10 per cent. of its weight of potassium chlorate, and heat this mixture upon a water-bath until evolution of ammonia has ceased. Then stir continuously and add pure concentrated hydrochloric acid (sp. gr. 1.19), 5 cc. every 5–10 minutes (25–40 cc. in all), and continue heating until evolution of chlorine has ceased. This usually requires about 40 minutes. Now add to the mixture 10–20 cc. of pure nitric acid of 40° Bé (62–63 per cent. HNO_3). Stir from time to time and allow the

[1] Use of electrolytic hydrochloric acid (see page 500) avoids the necessity of this purification. Tr.

[2] J. Vintilesco: Observations upon Some Methods of Destroying Organic Matter in the Detection of Metallic Poisons. Choice of a General Analytical Method. Bull. de Chim. 17 (1915), 99.

dish to remain upon the water-bath until most of the acid has evaporated. By this procedure only about 10 per cent. of organic material is not destroyed, that is, undissolved, consisting mostly of fat and fatty acids. The filtered liquid usually is yellowish and is excellently adapted for detection of metallic poisons by the general methods of analysis.

Destruction of Organic Matter by Free Chloric Acid

Sonnenschein and Jeserich[1] use pure chloric acid instead of potassium chlorate. Stir the finely divided material with water until the mass is thin and then transfer to a large flask together with a few cc. of chloric acid. Warm slowly and cautiously upon the water-bath. As soon as the mass swells and becomes porous, gradually add small portions of hydrochloric acid. Even a considerable quantity of cadaveric material will dissolve in 2–3 hours. Complete destruction of organic matter may be recognized by the fact that the upper layer of fatty acids is almost white and the liquid below nearly clear and yellowish. Water lost by evaporation should be replaced from time to time, otherwise the reaction may proceed with explosive violence. In other respects the mixture should be treated as in the procedure with potassium chlorate and hydrochloric acid.

Mai's[2] Method of Destroying Organic Matter

Mix finely divided material with dilute hydrochloric acid (1:12) until thin. Add a little potassium chlorate and heat over a free flame, adding from time to time small quantities of potassium chlorate (0.2 gram). Cool as soon as liquefaction of the mass is complete. Fat separates and usually can be easily removed from the liquid. Heat this fat once or twice with very dilute nitric acid, filter and add the filtrate to the main part of the liquid. Continue heating the latter, adding small quantities of ammonium persulphate, until the liquid is clear and yellowish white. Filter and saturate the filtrate as usual with hydrogen sulphide.

Ammonium persulphate is a powerful oxidizing agent which upon evaporation of its aqueous solution is decomposed into ammonium sulphate, sulphuric acid and oxygen:

$$(H_4N)_2S_2O_8 + H_2O = (H_4N)_2SO_4 + H_2SO_4 + O.$$

As compared with many other oxidizing agents, it possesses the great advantage of not adding non-volatile substances to the material under examination.

Other methods of destroying organic matter are given in Chapter V of this book (see pages 486 to 514).

EXAMINATION OF FILTRATE FOR METALLIC POISONS

Precipitation by Hydrogen Sulphide

A solution properly prepared by the Fresenius-v. Babo or by any other method, freed from excess of hydrochloric acid and filtered,

[1] P. Jeserich: Repertorium der analyt. Chem. 2 (1882), 379.

[2] C. Mai: Critical Operations in the Field of Forensic Chemistry. Zeitschr. f. Unters. d. Nahrgs.-u.Genussm. usw. 5 (1902), 1106.

should have only a faint yellow color.[1] Heat such a solution in a flask upon the water-bath and saturate with arsenic-free hydrogen sulphide.[2] Pass hydrogen sulphide for 0.5–1 hour, or longer, into the hot solution and continue this treatment after the solution has been removed from the water-bath and is cold.[3]

Allow the solution saturated with hydrogen sulphide to stand in the loosely stoppered flask for several hours, or better until the

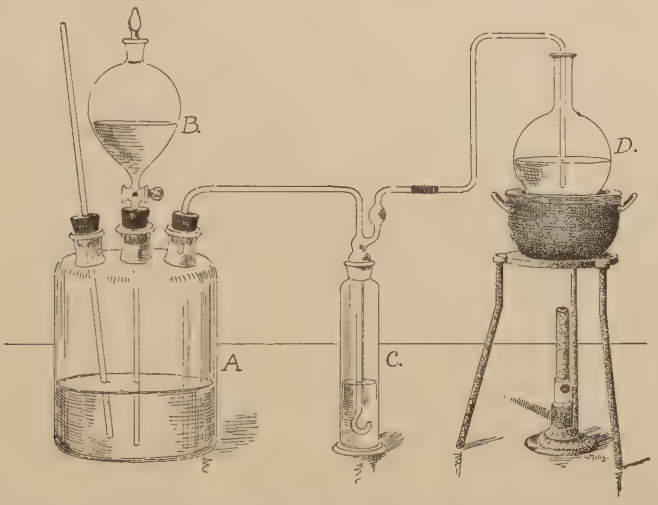


FIG. 18.—Apparatus for generating arsenic-free hydrogen sulphide. (a) Generator with dilute sulphuric acid; (b) Separating funnel with NaSH; (c) Wash-bottle; (d) Hydrochloric acid solution of material.

following day. If the solution then smells of hydrogen sulphide and blackens a piece of lead paper held over it, the next step in the process may be taken. Otherwise, warm the solution once more

[1] Chromium in not too small quantity imparts more or less of a green color both to solution and filtrate from the hydrogen sulphide precipitate, owing to presence of chromic chloride ($CrCl_3$).

[2] Prepare arsenic-free hydrogen sulphide by saturating dilute sodium hydroxide solution with hydrogen sulphide from crude iron sulphide and commercial hydrochloric acid. Pour this sodium hydro-sulphide (NaSH) solution into a separating funnel and add slowly to dilute sulphuric acid (1:4). Hydrogen sulphide is given off at once and can be regulated at will. It is practically arsenic-free and is especially suited to most forensic cases. The apparatus required is shown above in Fig. 18.

[3] In laboratory experiments, treatment with hydrogen sulphide may be somewhat shortened. A Kipp generator in which the gas is prepared from iron sulphide and hydrochloric acid may be used.

upon the water-bath and again saturate with hydrogen sulphide. Finally collect the hydrogen sulphide precipitate upon a small paper and wash with hydrogen sulphide water.

Examine the precipitate, produced by hydrogen sulphide, for arsenic, antimony, tin, mercury, lead, copper, bismuth and cadmium (Metallic Poisons I and II), and the filtrate from this precipitate for chromium and zinc (Metallic Poisons III, page 260).

Notes.—The fact should not be overlooked that vegetable and animal substances, consequently cadaveric material, when treated with hydrochloric acid and potassium chlorate, frequently give liquids, yielding yellow-red, brown-red or dark brown precipitates[1] with hydrogen sulphide even in absence of metals of this group. Such precipitates probably consist in part of organic sulphur compounds. Consequently in toxicological examinations, if hydrogen sulphide produces such a colored precipitate in acid solution, it should never be interpreted to mean that a metallic poison is positively present. Moreover, without further examination it is impossible to decide from the color of the hydrogen sulphide precipitate as to the presence of a particular metal.

Complete Precipitation.—Before testing for chromium and zinc in the filtrate from the hydrogen sulphide precipitate, add about 10 times the volume of strong hydrogen sulphide water to a portion of the filtrate, shake and allow to stand for several minutes. Unless a colored precipitate appears, the metals in question (Metallic Poisons I and II) have been completely removed, and the filtrate may then be further tested for chromium and zinc (Metallic Poisons III). Otherwise, first strongly dilute the entire filtrate from the hydrogen sulphide precipitate with water and again saturate with hydrogen sulphide. In presence of much hydrochloric acid, lead and cadmium are incompletely precipitated by hydrogen sulphide.

Extraction of Hydrogen Sulphide Precipitate with Ammonia-Ammonium Sulphide

Thoroughly extract the washed hydrogen sulphide precipitate, while moist, upon the filter with a hot mixture of approximately equal parts of ammonia and yellow ammonium sulphide. This may be accomplished by heating 5–10 cc. of the mixture of ammonia and ammonium sulphide to boiling and dropping the solution over the

[1] Repeated treatment with potassium chlorate and hydrochloric acid dissolves thoroughly washed casein and fibrin almost completely, giving a filtrate from which hydrogen sulphide precipitates dirty yellow to brownish substances. Such precipitates are amorphous and contain together with much free sulphur organic sulphur compounds, possibly thio-aldehydes and ketones.

precipitate upon the filter. Reheat the filtrate and pour again over the precipitate. Repeat this operation several times. Finally wash the filter with a few cc. of fresh ammonia-ammonium sulphide mixture.

Test the entire filtrate for arsenic, antimony, tin and copper[1] (Metallic Poisons I) and the residue left upon the filter for mercury, lead, copper, bismuth and cadmium (Metallic Poisons II).

METALLIC POISONS I

Arsenic, Antimony, Tin, Copper

Examination of Hydrogen Sulphide Precipitate Soluble in Ammonia-Ammonium Sulphide

Use the solution prepared as described by treating the hydrogen sulphide precipitate with a hot mixture of ammonia and yellow ammonium sulphide. This solution is usually dark brown owing to dissolved organic substances.[2] Evaporate the solution to dryness in a porcelain dish upon the water-bath. Moisten the cold residue with fuming nitric acid and again evaporate. Then intimately mix the residue with about three times the quantity[3] of a mixture of 2 parts of sodium nitrate and 1 part of dry sodium carbonate. Thoroughly dry this mixture upon the water-bath and introduce small portions at a time into a porcelain crucible containing a little fused sodium nitrate heated to redness. After the final addition, heat the

[1] Copper sulphide (CuS) is soluble to considerable extent in hot yellow ammonium sulphide. An ammonium sulphide solution containing copper, treated as described on page 250, yields copper oxide which gives the melt a more or less gray or black appearance. If the melt is extracted with water, the residue contains black copper oxide with stannic oxide and sodium pyroantimonate. To detect copper, dissolve the black residue in a little hot dilute hydrochloric acid and divide the solution into two parts. Add ammonia to one part until alkaline. The solution is blue, if copper is present. Add potassium ferrocyanide to the other part. A brownish red precipitate of cupric ferrocyanide ($Cu_2Fe(CN)_6$) will appear, if copper is present.

[2] In toxicological examinations, even in absence of metals, hydrogen sulphide produces in the hydrochloric acid solution in most cases dark colored precipitates (see above). The latter are due to organic substances always present and usually soluble with a deep dark brown color in the hot ammonia-ammonium sulphide mixture.

[3] In most laboratory experiments, 3 grams of a mixture of 2 grams of sodium nitrate and 1 gram of sodium carbonate are sufficient. Too great an excess of sodium nitrate should be avoided.

crucible a short time, introducing possibly a little more sodium nitrate, until the fused mass is colorless. In presence of copper the melt is gray, or grayish black, from copper oxide. Sodium arsenate, sodium pyro-antimonate, sodium stannate, as well as stannic oxide and copper oxide, may also be present. Soften the cold melt with hot water and wash into a flask. Add a little sodium bicarbonate to the clear or cloudy liquid, to decompose the small quantity of sodium stannate, possibly in solution, and precipitate all the tin as stannic oxide and filter.

Any arsenic present will be in the filtrate (A) as sodium arsenate (Na_2HAsO_4) and the residue upon the filter (B) may contain antimony as sodium pyro-antimonate ($Na_2H_2Sb_2O_7$), tin as the oxide (SnO_2), as well as copper oxide.[1]

Examination of Filtrate A for Arsenic

In toxicological examinations proof of the presence of arsenic in general is afforded by isolating this poison in its elementary condition and thereby removing all doubt as to its existence in the given material. Two methods are in use for this purpose, namely, the Berzelius-Marsh and the Fresenius-v. Babo method. Both are very accurate and exclude all confusion of arsenic and antimony.

Chapter V of this book gives the details of the very delicate biological test for detecting arsenic, requiring the use of certain moulds; the electrolytic separation of arsenic at the cathode as arsine; and the arsenic tests of Bettendorff, Gutzeit and Reinsch. In the same part of this book, isolation of arsenic as trichloride by the Schneider-Fyfe-Beckurts method and its colorimetric estimation have been taken up.

1. Berzelius-Marsh Method

Principle.—Nascent hydrogen converts oxygen compounds of arsenic, arsenious and arsenic acid, as well as their salts into arsine (AsH_3):

$$(\alpha)\ H_3AsO_3 + 6H = AsH_3 + 3H_2O,$$
$$(\beta)\ H_3AsO_4 + 8H = AsH_3 + 4H_2O.$$

At red heat arsine is decomposed into metallic arsenic and hydrogen:

$$AsH_3 = As + 3H.$$

This equation expresses formation of the arsenic mirror.

[1] Even in absence of the substances mentioned under B, a small insoluble residue usually appears. This may come from the porcelain crucible, the glazing of which is slightly attacked in the fusion with sodium nitrate and carbonate.

Hydrogen containing arsine burns with a bluish white flame to arsenic trioxide and water (α). Depress a piece of cold porcelain upon this flame. Only hydrogen burns, whereas arsenic is deposited as the metal (β). This is the so-called arsenic spot:

$$(\alpha)\ 2AsH_3 + 3O_2 = As_2O_3 + 3H_2O,$$
$$(\beta)\ 2AsH_3 + 3O = 2As + 3H_2O.$$

Hydrogen containing arsine, passed into dilute silver nitrate solution, precipitates black metallic silver. At the same time arsenious acid passes into solution:

$$AsH_3 + 3H_2O + 6AgNO_3 = H_3AsO_3 + 6HNO_3 + 6Ag.$$

Procedure.—Acidify the solution of the melt, that is, "Filtrate A," prepared as described above (page 250) and possibly containing sodium arsenate, with dilute arsenic-free sulphuric acid. Evaporate this solution in a porcelain dish upon an asbestos plate over a small flame. Add 15–20 drops of concentrated sulphuric acid, to expel completely any nitric acid present in the residue, and heat until copious white fumes of sulphuric acid are given off. The residue[1] in the dish is a thick, colorless liquid having a strong acid reaction. Arsenic, if present, is in the form of arsenic acid (H_3AsO_4) and when cold frequently solidifies to a white crystalline mass. Examine the solution of this residue for arsenic in the Marsh apparatus. This solution is also well adapted for the electrolytic detection of arsenic (see pages 518 and 524).

Marsh Apparatus.—Place 15–25 grams of pure arsenic-free zinc[2] (granulated or in small rods) in the reduction-flask A of the Marsh apparatus (Fig. 19). Pour cold dilute arsenic-free sulphuric acid through the thistle-tube upon the zinc. This acid should contain 15–16 per cent. of H_2SO_4.[3] Evolution of hydrogen should not be too rapid, otherwise the liquid in the evolution-flask may get too warm. The result may be reduction of some sulphuric acid to sul-

[1] To insure complete removal of nitric acid, test a few drops of this residue by mixing with concentrated sulphuric acid and carefully adding to a cold saturated solution of ferrous sulphate.

[2] The passage of hydrogen from 15–20 grams of zinc, treated with dilute arsenic-free sulphuric acid, through the strongly heated ignition-tube C of the Marsh apparatus should not give a trace of arsenic after 1 hour. Such zinc is practically arsenic-free and is suited for forensic-chemical examinations. Bertha spelter from the New Jersey Zinc Company will meet this test.

[3] Add 1 volume of pure arsenic-free concentrated sulphuric acid to 5 volumes of distilled water. This acid when cold is suitable for use in the Marsh test.

phurous acid and even to hydrogen sulphide, more or less interfering with or entirely preventing detection of arsenic. Therefore place reduction-flask A in a dish of cold water, if the acid becomes too warm. In using the Marsh apparatus, the following precautions should be taken:

1. Make sure that the apparatus is absolutely tight.

2. Expel air completely before igniting hydrogen. To tell when this point is reached, collect hydrogen in a dry test-tube until it ignites without detonation when carried to a flame. When hydrogen stands this test, ignite the gas at the end of ignition-tube C.

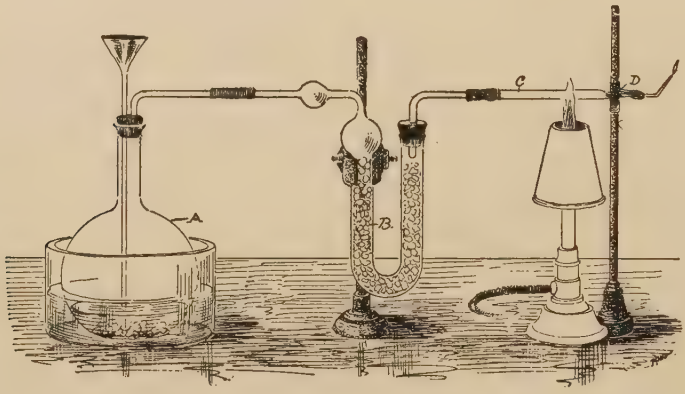


FIG. 19.—Marsh apparatus for the detection of arsenic and antimony. (a) Hydrogen generator; (b) Calcium chloride drying-tube; (c) Hard glass tube; (d) Arsenic mirror.

There is then no danger of an explosion within the apparatus. If the apparatus is tight and evolution of hydrogen is not too rapid, it requires about 5–8 minutes to expel the air.

3. Test hydrogen to insure entire freedom from arsenic. Neither arsenic mirror nor spot should appear.

If hydrogen is arsenic-free, gradually introduce the perfectly cold sulphuric acid solution, containing arsenic as arsenic acid, in small portions into reduction-flask A. At the same time heat ignition-tube C to redness just back of the capillary tube. If the solution contains arsenic, the gas generated consists of a mixture of arsine (AsH_3) and hydrogen. A shining mirror of metallic arsenic appears, often in a few minutes, just beyond the point of ignition. Traces of arsenic require considerable time before a brown or brown-

ish black film appears. A piece of white paper held behind the tube brings out clearly even a minute arsenic mirror.

Remove the flame from ignition-tube C. If arsenic is present, the hydrogen flame will become bluish white. At the same time white fumes of arsenic trioxide (As_2O_3) will arise from the flame.

To produce the lustrous, brownish black spot (arsenic spot), depress a cold porcelain dish upon the hydrogen flame.

Arsine has an exceedingly characteristic, garlic-like odor. Extinguish the hydrogen flame and allow the gas to escape.[1] The odor is evident even when hydrogen contains only traces of arsine.

A third method of detecting arsenic by the Marsh apparatus consists in extinguishing the hydrogen flame and passing the gas into dilute neutral silver nitrate solution. Arsine will darken this solution, producing a black precipitate of metallic silver. The solution contains arsenious acid and free nitric acid.

Filter through a double paper to remove silver and carefully neutralize the filtrate with a few drops of very dilute ammonium hydroxide solution. If the solution is neutral, it is possible to obtain a yellowish white precipitate of silver arsenite (Ag_3AsO_3) but this compound dissolves easily in ammonium hydroxide solution and in nitric acid.

Gutzeit's Arsenic Test.—Extinguish the flame at the end of reduction-tube C and hold over the tube a strip of paper moistened with concentrated silver nitrate solution (1:1). A yellow stain will appear, if hydrogen contains arsine. A drop of water added to this yellow spot will turn it black.

In many cases it is advisable to pass through the Marsh apparatus during the entire test a moderate stream of hydrogen generated in a Kipp apparatus from arsenic-free zinc and dilute sulphuric acid. The gas should also be washed through water before it enters the Marsh apparatus. The arrangement of such an apparatus is shown in Fig. 20.

Differences between Spots and Mirrors of Arsenic and Antimony

Nascent hydrogen reduces various antimony compounds ($SbCl_3$, Sb_2O_3, $HSbO_3$, $KSbOC_4H_4O_6$, etc.), producing the colorless gas stibine (SbH_3). The behavior of this compound in the Marsh apparatus closely resembles that of arsine, for it gives a spot and mirror but produces a black precipitate of silver

[1] Detection of arsine is so easy by other tests that it seems somewhat superfluous to confirm its presence in this way in view of its very poisonous properties. Tr.

antimonide (Ag_3Sb) instead of metallic silver, when passed into silver nitrate solution.

Although the procedure described separates arsenic from antimony and excludes possibility of these two metals appearing together in the Marsh test, it seems advisable to point out the differences between them, especially as it is always necessary to identify arsenic mirrors and spots by other tests. In many cases, particularly when tests have indicated presence of antimony, the mirror and spot of this metal should be produced in the Marsh apparatus with the liquid suspected to contain antimony, for the purpose of confirming the other tests.

Differences between spots and mirrors of arsenic and antimony are as follows:

1. The arsenic mirror has a high metallic luster. It is brownish black and easily volatile. Owing to the latter property, it sublimes when heated in a stream of hydrogen. In case of the antimony mirror, which appears on both sides of the flame, the metal in contact with the heated glass fuses and is silver-

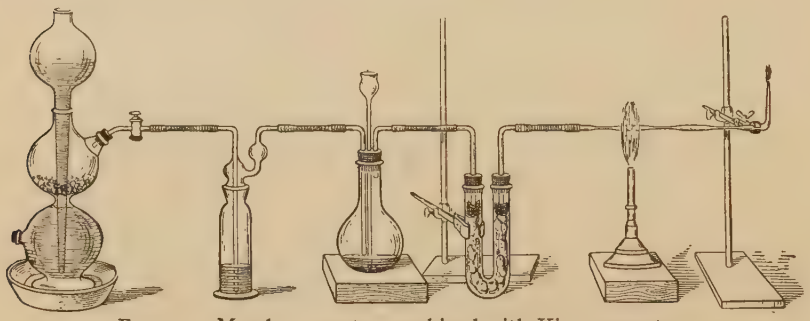


FIG. 20.—Marsh apparatus combined with Kipp generator.

white. But in places removed from the flame it is almost black and has hardly any luster. Stibine decomposes at a temperature much below that required for arsine. This fact explains deposition of this metal on both sides of the flame. Antimony volatilizes at a high temperature and consequently sublimes with difficulty.

2. The arsenic spot, if not too heavy, is brownish black or brown and lustrous. It dissolves readily in sodium hypochlorite solution, forming arsenious and arsenic acid:

$$3H_2O + 2As + 3NaOCl = 2H_3AsO_3 + 3NaCl.$$

The antimony spot is dull, velvet-black and without luster. A thin film of antimony is never brown but has a dark, graphite-like appearance. It is insoluble in sodium hypochlorite solution.

3. A drop of concentrated nitric acid, or moist chlorine, at once dissolves the arsenic spot forming arsenic acid. Neutralize with ammonia and add silver nitrate solution. A reddish precipitate of silver arsenate (Ag_3AsO_4) will appear.

Nitric acid, or moist chlorine, also dissolves the antimony spot but silver nitrate does not produce a colored precipitate.

4. Gently heat the ignition-tube and pass a stream of dry hydrogen sulphide over the arsenic mirror. Yellow arsenic trisulphide (As_2S_3) will appear. The antimony mirror becomes brown-red to black (Sb_2S_3).

5. Arsine and stibine are differentiated by their behavior toward dilute silver nitrate solution. Both produce black precipitates. Arsine precipitates metallic silver and the filtrate contains arsenious acid. On the other hand, stibine precipitates silver antimonide (Ag_3Sb) and the filtrate does not contain a trace of antimony. To detect antimony, filter off the black precipitate, wash and boil for some time with 10–15 per cent. tartaric acid solution. Antimony is completely dissolved, whereas silver remains as a grayish white residue. Add dilute hydrochloric acid to this solution and then treat with hydrogen sulphide. Antimony will appear as orange-red antimony sulphide.

2. Fresenius-von Babo Method

Principle.—Fusion of oxygen and sulphur compounds of arsenic with a mixture of sodium carbonate and potassium cyanide causes reduction with formation of an arsenic mirror. As a result potassium cyanide is converted into potassium cyanate or sulphocyanate:

$$As_2O_3 + 3KCN = As_2 + 3KCNO,$$
$$As_2S_3 + 3KCN = As_2 + 3KSCN.$$

Procedure.—Use for this test the aqueous, sulphuric acid solution (A), prepared by the method already described and containing arsenic in the form of

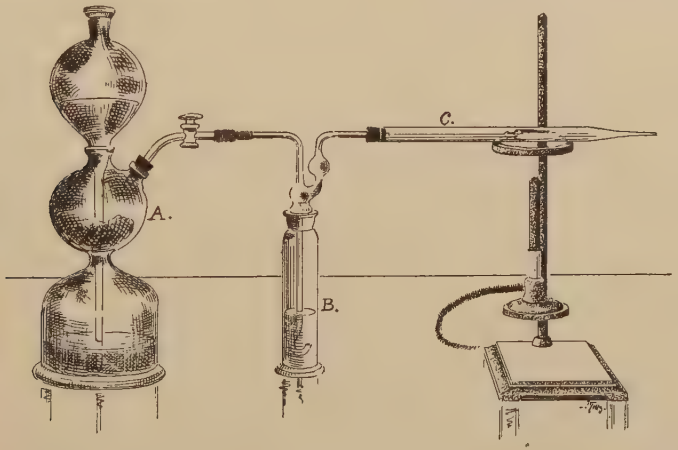


FIG. 21.—Fresenius-von Babo apparatus. (a) Carbon dioxide generator; (b) Drying-bottle with pure, concentrated sulphuric acid; (c) Ignition-tube and boat.

arsenic acid (page 251). To reduce arsenic acid to arsenious acid, add a few cc. of sulphurous acid to the solution and heat until odor of this acid has entirely disappeared. Dilute this solution with water and treat with hydrogen sulphide. Collect the precipitate (As_2S_3) upon a small filter and wash thoroughly. Dis-

solve the precipitate upon the filter in a little hot ammonium hydroxide solution. Evaporate this solution in a porcelain dish upon the water-bath and heat the residue with concentrated nitric acid. Expel the latter completely by evaporation, moisten the residue with a little water, and add enough dry sodium carbonate to render the mixture distinctly alkaline. Dry thoroughly upon the water-bath and triturate the residue in a mortar with several times the quantity of a mixture of 3 parts of dry sodium carbonate and 1 part of pure potassium cyanide.

Transfer this mixture to a porcelain boat and place in an ignition-tube of hard glass. Heat in a stream of carbon dioxide dried by means of arsenic-free sulphuric acid (Fig. 21). To expel moisture, first heat the ignition-tube gently where the boat is and then ignite at a bright red heat. A mirror of arsenic will appear upon the cooler part of the tube, if arsenic is present.

A simpler method of detecting arsenic by potassium cyanide is often used Heat the thoroughly dried material containing arsenic (As_2O_3, As_2S_3) in a bulb-tube with a dry mixture of sodium carbonate and potassium cyanide until fusion takes place. If the tube is smaller above the bulb, the arsenic mirror will form in the constricted area (Fig. 22).

FIG. 22.—(A) Substance and fusion-mixture; (B) Arsenic mirror.

Examination of Residue B for Antimony, Tin and Copper

Residue B (see page 250), insoluble in water and obtained by fusion, may contain sodium pyro-antimonate, stannic and cupric oxide. Treat this residue upon the filter with a little hot dilute hydrochloric acid (equal parts of concentrated acid and water). Pass this acid repeatedly through the paper until most of the residue is dissolved. If the original color of residue B and melt was gray or black, first examine a portion of the hydrochloric acid solution for copper. Excess of ammonia will produce a blue color. Potassium ferrocyanide solution will give a brownish red precipitate, or only a coloration with traces of copper.

Concentrate the remainder of the hydrochloric acid solution to a few drops in a porcelain dish upon the water-bath, and put two drops of this solution upon platinum foil in contact with zinc. Antimony will produce a black, tin a grayish and copper a dark reddish brown spot upon platinum. There is little chance of confusing the tin or copper spot with that given by antimony.

Dilute the remainder of the hydrochloric acid solution of residue B with water and introduce a piece of zinc. Keep the zinc in the solution as long as hydrogen is evolved. Collect black, metallic flocks from this operation upon a small filter. Wash thoroughly and gently warm with a little concentrated hydrochloric acid. Finally filter the solution. Antimony does not dissolve, whereas tin passes into solution as stannous chloride ($SnCl_2$) and is in the filtrate. It may be recognized by the following tests:

(a) **Mercury Test.**—Add a few drops of mercuric chloride solution to a portion of the filtrate. Tin will precipitate white mercurous chloride. In addition, heat will produce gray, metallic mercury, if stannous chloride is present in large excess.

(b) **Prussian Blue Test.**—Add a few drops of a dilute mixture of ferric chloride and potassium ferricyanide solution to a second portion of the filtrate. Tin will produce a precipitate of Prussian blue.

This test is not characteristic of tin, for many other substances capable of reducing ferri-ferricyanide to ferri-ferrocyanide, that is, to Prussian blue, act in the same way.

For further identification of antimony, dissolve black flocks, insoluble in hydrochloric acid, in a few drops of hot aqua regia. Expel excess of acid upon the water-bath and dilute the residue with water. If the quantity of antimony is not too small, water will precipitate white antimony oxychloride (SbOCl). Redissolve this precipitate in a little dilute hydrochloric acid. Test a portion of this solution for antimony with hydrogen sulphide. Introduce the remainder into the Marsh apparatus and produce the antimony spot and mirror, or test for stibine with silver nitrate solution as described (see page 255).

METALLIC POISONS II

Mercury, Lead, Copper, Bismuth, Cadmium

Examination of Hydrogen Sulphide Precipitate Insoluble in Ammonium Sulphide

That portion of the hydrogen sulphide precipitate, insoluble in ammonium sulphide, may contain sulphides of mercury, lead, copper, bismuth and cadmium. Examine this precipitate according to the general methods employed in qualitative analysis. If there is only a slight precipitate, treat it repeatedly upon the filter with a few cc.

of warm, rather dilute nitric acid (1 volume of concentrated acid and 2 volumes of water) by heating the filtrate each time to boiling and pouring it over the precipitate. Mercuric sulphide does not dissolve (A), whereas sulphides of lead, copper, bismuth and cadmium pass into solution as nitrates (B).

A. Examination of Residue upon Filter for Mercury

A residue, remaining after extraction with hot nitric acid of the portion of the hydrogen sulphide precipitate insoluble in ammonium sulphide, should always be tested for mercury, even when it is not pure black or gray-black! Treat this residue upon the filter with a little hot, somewhat diluted hydrochloric acid, containing in solution a few crystals of potassium chlorate, and pass the acid through the paper several times. Evaporate the filtrate to dryness in a porcelain dish upon the water-bath, and dissolve the residue in 2–3 cc. of water containing hydrochloric acid. Filter this solution and examine the filtrate for mercury:

(a) **Stannous Chloride Test.**—Add a few drops of stannous chloride solution to a portion of the filtrate. If mercury is present, a white precipitate of mercurous chloride will appear. Excess of stannous chloride, especially if heat is applied, will reduce this precipitate to gray, metallic mercury.

(b) **Copper Test.**—Put a few drops of the filtrate upon a small piece of bright copper. Mercury immediately deposits a gray spot having a silvery luster when rubbed. Wash the copper, upon which mercury is deposited, successively in water, alcohol and ether. Dry thoroughly in the air and heat in a small bulb-tube of hard glass. Mercury will sublime and collect in small, metallic globules on the cool sides of the tube. A trace of iodine vapor, introduced into the tube, immediately converts the gray sublimate into scarlet mercuric iodide (HgI_2).

(c) **Phosphorous Acid Test.**—Add some phosphorous acid to another portion of the filtrate and warm gently. If mercury is present, a white precipitate of mercurous chloride[1] will appear.

(d) **Mercuric Iodide Test.**—Add 1–2 drops of very dilute potassium iodide solution to the remainder of the filtrate. A red precipitate (HgI_2), readily soluble in excess of potassium iodide, shows presence of mercury.[2]

[1] $2HgCl_2 + H_2O + H_3PO_3 = 2HgCl + 2HCl + H_3PO_4$.

[2] (α) $HgCl_2 + 2KI = HgI_2 + 2KCl$. ($\beta$) $HgI_2 + 2KI = K_2^{++}(HgI_4)^{=}$.

B. Examination of Nitric Acid Solution

The nitric acid solution may contain nitrates of lead, copper, bismuth and cadmium. Evaporate this solution in a porcelain dish upon the water-bath nearly to dryness and dissolve the residue in a little hot water. Add dilute sulphuric acid to the filtered, clear solution. If lead is present, a heavy white precipitate of lead sulphate, soluble in basic ammonium tartrate, will appear. Test the filtrate from the precipitate of lead sulphate, or the solution remaining clear upon treatment with sulphuric acid, next for copper and bismuth:

(a) **Copper and Bismuth Tests.**—Add ammonia in excess to the greater part of the filtrate. It will produce a blue color, if copper is present. A white precipitate at the same time may be bismuthous hydroxide.[1] For the positive detection of bismuth, wash the precipitate and dissolve upon the filter in a few drops of hot, dilute hydrochloric acid. Pour this solution into considerable water. A white precipitate of bismuthous oxychloride (BiOCl) proves presence of bismuth.

For additional proof of bismuth, redissolve the white precipitate in dilute hydrochloric acid and divide the solution into two parts. Add a few drops of stannous chloride solution to one part and then sodium hydroxide solution until the reaction is strongly alkaline.[2] This will produce a black precipitate of metallic bismuth. Add a few drops of sulphurous acid and potassium iodide solution to the second part of the hydrochloric acid solution. If bismuth is present, an intense orange-yellow or only a yellowish color will appear. This is a very delicate test, making it possible to detect with certainty 0.01 mg. of bismuth in 1 cc. of solution.

(b) **Potassium Ferrocyanide Test.**—Add potassium ferrocyanide solution to another portion of filtrate from lead sulphate, or solution remaining clear upon treatment with sulphuric acid. According to the quantity of copper present, a brown-red precipitate of copper ferrocyanide ($Cu_2Fe(CN)_6$) immediately appears, or only a brown-red coloration yielding flocks of the same color upon standing.

[1] Ammonium hydroxide solution does not precipitate pure bismuthous hydroxide ($Bi(OH)_3$) from solutions of bismuth salts but a basic salt, the composition of which depends upon temperature and concentration of the solutions.

[2] If stannous chloride solution produces in acid solution a gray or black precipitate, it is due to mercury; bismuth is reduced only in alkaline solution by the stannous salt, that is, by sodium stannite ($Sn(ONa)_2$).

(c) **Precipitation of Metallic Copper.**—Put a bright knife-blade, or iron nail, in the solution to be tested for copper. If copper is present, a red film of copper will be deposited upon the blade or nail.

To detect cadmium in presence of copper, add potassium cyanide to the blue solution in (a) produced by excess of ammonia, or to the blue filtrate from bismuthous hydroxide, until the color is discharged and then pass in hydrogen sulphide. Cadmium is precipitated as yellow sulphide (CdS), whereas copper will remain in solution as colorless potassium cuprocyanide ($K_6Cu_2(CN)_8$).

When copper is absent, test for cadmium direct by passing hydrogen sulphide into the ammoniacal solution obtained in (a). If a reddish or brownish instead of a yellow precipitate appears, filter and heat the dried precipitate upon charcoal in the blow-pipe flame. Cadmium will give a brown coating.

METALLIC POISONS III

Examination for Chromium and Zinc

Test the filtrate from the hydrogen sulphide precipitate (page 248) for chromium and zinc. Concentrate the filtrate in a porcelain dish to about one-third its original volume and divide this solution into two parts.

To test for zinc, add enough ammonia to render one-half the concentrated filtrate alkaline. This treatment usually gives the solution a dark color. Then add ammonium sulphide solution in excess. This reagent almost always produces a precipitate even when zinc is absent, for solutions prepared from animal and vegetable materials usually contain iron compounds and phosphates of the metals of the earths and alkaline earths. When this precipitate has settled, add acetic acid until the solution has a faint acid reaction. Stir the mixture thoroughly and allow it to stand for some time. The color of the precipitate becomes lighter because acetic acid partially dissolves sulphide of iron. Moreover phosphates are partially dissolved, except ferric phosphate ($FePO_4$) which is insoluble in acetic acid. Collect the precipitate upon a filter, wash, dry and ignite precipitate and filter in a porcelain crucible. Before ignition, moisten the filter with a concentrated solution of ammonium nitrate to prevent reduction of zinc compounds, when ignited with carbon from the burning filter, to metallic zinc and volatilization of the latter. Extract the residue from ignition with dilute sulphuric acid, filter and divide the filtrate into two parts.

(a) Add sodium hydroxide solution in excess to one portion of the filtrate and shake thoroughly. Filter, to remove the white precipitate of ferric phosphate that almost always appears, add a few drops of ammonium or hydrogen sulphide solution to the clear filtrate and warm gently. Zinc, if present, gives a white, flocculent precipitate of zinc sulphide.

(b) Add ammonia in excess to the second part of the filtrate. Filter, to remove any precipitate that appears, acidify the filtrate with acetic acid, warm and pass in hydrogen sulphide. Zinc, if present, will appear as the white, flocculent sulphide.

(c) As an additional test for zinc, dissolve in a few drops of hot dilute hydrochloric acid the precipitate produced by ammonium or hydrogen sulphide (as described above in *a* and *b*), after it has been collected upon a filter and washed. Boil for some time to expel hydrogen sulphide and filter to remove precipitated sulphur. Add potassium ferrocyanide solution to the clear, cold filtrate. Zinc ferrocyanide ($Zn_2Fe(CN)_6$) is precipitated white, slimy and nearly insoluble in dilute hydrochloric acid.[1]

To test for chromium[2] in the second part of the filtrate from the hydrogen sulphide precipitate, concentrate the solution in a porcelain dish to a small volume. Then add twice the quantity of potassium nitrate and sodium carbonate until the reaction is strongly alkaline and heat until perfectly dry. Add this dry residue in small portions to a little potassium nitrate fused in a crucible. In fusing a large quantity of material, it is advisable to use a large, bright nickel crucible which is especially adapted for this operation. When fusion is complete and all organic matter is oxidized, cool, boil crucible and contents in a porcelain dish with not too much water, and filter the solution. If the filtrate contains chromium, it will have a more or less intense yellow color. Even traces of chromium[3] may be

[1] Excess of potassium ferrocyanide combines with zinc ferrocyanide first precipitated, forming insoluble potassium zinc ferrocyanide:

$$3Zn_2Fe(CN)_6 + K_4Fe(CN)_6 = 2K_2Zn_3(Fe(CN)_6)_2.$$

[2] In testing for metallic poisons, chromic oxide (Cr_2O_3), insoluble in acids, may be disregarded as it is not poisonous.

[3] Two drops of 10 per cent. potassium chromate solution (=0.01 gram of K_2CrO_4) in 500 cc. of water produce a marked yellow color. Fifty cc. of this solution, containing 1 mg. of K_2CrO_4, have a yellow color that can still be recognized with certainty.

recognized by the yellow color of the filtrate. When the solution of the melt is colorless, it is unnecessary to test for chromium. To detect chromium with certainty, when the filtrate is yellow, divide the solution into two portions and make the following tests:

(a) **Chrome Yellow Test.**—Add acetic acid in excess to one portion of the filtrate, boil for some time to expel carbon dioxide and nitrous acid, and add a few drops of lead acetate solution. A yellow precipitate of lead chromate ($PbCrO_4$) will appear, if chromium is present. When the precipitate is mixed with considerable lead sulphate or chloride, the color is only yellowish. A white precipitate is due to $PbSO_4$, $PbCl_2$ or $Pb_3(PO_4)_2$. When the aqueous solution of the melt is colorless, such a precipitate is usually obtained.

If solutions of potassium nitrite, always formed in potassium nitrate fusions, lead acetate and acetic acid are mixed, the resulting solution has a strong yellow color. A white precipitate in such a solution may appear to have a yellow color. To eliminate this source of error, allow the precipitate to settle, collect upon a filter, and wash thoroughly. If it is pure white, chromium is absent.

(b) **Reduction Test.**—Add sulphurous acid to the second portion of the yellow filtrate. The yellow color will change to green, or green-blue, with formation of chrome alum. This is not as delicate as the preceding test.

METALLIC POISONS IV

Examination of Residue from Hydrochloric Acid and Potassium Chlorate for Barium, Lead, and Silver

Wash thoroughly with water the residue, undissolved after treatment with hydrochloric acid and potassium chlorate, and dry in an air-closet or upon a porous plate. Then add three times the quantity of a mixture of 2 parts of potassium nitrate and 1 part of dry sodium carbonate and triturate in a mortar together with the filter. Gradually introduce this mixture into a hot porcelain crucible. In this operation organic substances (fat, fatty acids, etc.) are oxidized by potassium nitrate with considerable deflagration. Finally when all material is in the crucible, add 0.5 gram more of potassium nitrate. Cool the melt, soften with hot water, wash into a flask, and pass carbon dioxide for several minutes through the liquid which is usually turbid. This treatment converts caustic

alkali formed during fusion into carbonate and completely precipitates any lead that may be in solution. Then heat the solution to boiling and allow to settle for some time. Collect upon a small filter the deposit[1] which may contain barium carbonate, basic lead carbonate and metallic silver. The latter will impart a gray color to the deposit. Thoroughly wash the precipitate with hot water and dissolve upon the paper in hot, rather dilute nitric acid,[2] passing the acid through the paper several times. Evaporate this nitric acid solution to dryness in a porcelain dish. Dissolve the residue in water, heat the solution to boiling, and precipitate silver with dilute hydrochloric acid. Filter, to remove silver chloride, and pass hydrogen sulphide through the filtrate to precipitate lead. To test for barium in the filtrate from lead sulphide, or in the solution that gave no precipitate with hydrogen sulphide, heat to boiling to expel hydrogen sulphide and filter to remove insoluble matter. Then add dilute sulphuric acid which will precipitate barium sulphate. The following tests of identification should be applied to these several precipitates:

For the further identification of silver, dry the precipitate caused by hydrochloric acid and fuse in a porcelain crucible with a little potassium cyanide. Extract the cold melt with hot water. Metallic silver will remain undissolved.

To confirm presence of lead, dissolve the hydrogen sulphide precipitate in hot nitric acid, and evaporate the solution to dryness. Dissolve the residue in water, filter and test the solution for lead with dilute sulphuric acid, potassium chromate and potassium iodide.

As additional proof of presence of barium, collect the sulphuric acid precipitate upon paper, wash thoroughly and test upon a clean platinum wire in a non-luminous flame. Barium imparts a yellowish green color to the flame. To avoid any mistake, examine this flame with the spectroscope. In forensic-chemical examinations these reactions of identification should always be carried out with the utmost care!

[1] Even in absence of barium, lead and silver, such a deposit usually appears. In that case it probably consists of material from the porcelain crucible, the glazing of which is attacked to some extent in the process of fusion.

[2] Use 5–6 cc. of an acid prepared by mixing 1 volume of concentrated nitric acid with 2 volumes of water.

TOXIC ACTION OF ARSENIC, ANTIMONY AND METALLIC POISONS AND ELIMINATION FROM THE HUMAN ORGANISM

Absorption and Toxic Action of Arsenic

Arsenious and arsenic acid exert a strong corrosive action that manifests itself upon both the outer skin and mucous membranes. Rapid absorption of these arsenic compounds is ascribed to such action. How far-reaching absorption of arsenic through the outer skin is appears from the fact that fatal poisonings have repeatedly followed use of arsenic plaster. As a result of paralyses after absorption, blood-pressure falls and the abdominal organs are highly congested. This extends especially to the mucous surface of the gastro-intestinal tract, giving it a bright red appearance as in severe gastro-intestinal catarrh. This apparent gastro-enteritis is rendered more intense by the fact that absorbed arsenic is eliminated not only by the kidneys but also by the glands of the intestinal mucous membrane in consequence of which they are very severely irritated. Like phosphorus all arsenicals cause severe disturbances of metabolism, recognized by fatty degeneration of almost all organs of the vascular parenchyma of the kidneys, liver, cardiac muscle, and gastro-intestinal epithelium.

Brain and spinal cord also suffer severe injury. Disorders of the skin frequently appear. Such effects are explained by elimination of small quantities of arsenic in the skin which then give rise to oedema and pigmentation. Ill effects due to arsenic manifest themselves in mucous membranes as angina, hoarseness, cold, persistent bronchial catarrh and inflammation of the eyes. In case death does not occur too soon, arsenic finally is deposited in the bones. According to Kobert, this deposition may be regarded as a kind of natural cure, for all symptoms of poisoning disappear at this stage because arsenic deposited as calcium arsenate, $Ca_3(AsO_4)_2$, is redissolved only with difficulty.

According to Brouardel,[1] poisoning by arsenic usually takes place in four phases. In the first phase the gastro-intestinal tract and kidneys are attacked, in the second other abdominal organs, organs of the chest, and central nervous system. In the third phase skin, hair and nails are affected, and in the fourth the poison not eliminated is deposited in the bones as calcium arsenate and there fixed. In very rare cases of poisoning, the first series is said apparently to be entirely omitted so that symptoms of nervous paralysis appear at once. In most cases the first symptoms of poisoning do not appear for a half to a full hour. In the rarer, the paralytic form, uniform paralysis of the central nervous system and heart is the predominant symptom and may result in death within 10 hours. In the gastro-intestinal form, the manifestations of paralysis, as compared with gastro-intestinal symptoms, are very much suppressed. The effects noted are: emesis, great thirst, dryness and itching in mouth and throat, difficulty in swallowing, extremely violent pains in the abdomen, severe, foul-smelling diarrhoea having the appearance of rice-water or dysenteric. As a result secretion of the urine is diminished and it usually contains blood and cylinders and very soon

[1] Brouardel: Les intoxications. Paris 1904.

also arsenic and sugar. In the next place the phenomena that usually appear are those of the heart and nervous system, that is, headache, dizziness, pain in the limbs, low blood-pressure, weak irregular pulse, coldness of extremities, syncope, coma, convulsions, and general paralysis. In many cases disorders of the skin appear, having the character of an eczema or giving the picture of erysipelas with formation of little blisters, usually on the face and genitals. The contents of these blisters are said to contain arsenic. Even in milder skin affections general scaling of the skin together with loss of hair may appear. In addition, cases are described in the literature where only gastro-intestinal phenomena have been observed. Frequently this gastro-enteritis results in fatal collapse within 18–70 hours. In rarer cases death ensues more rapidly, within 10 hours, as in the paralytic form of arsenical poisoning. In not a few instances death comes on much more slowly, that is, after 4–14 days.

Lethal Dose.—According to Kobert, even 0.1–0.3 gram of white arsenic (As_2O_3) may produce fatal results. Arsenates are less toxic than arsenites. Elimination of arsenic in urine begins 6–12 hours after the arsenical preparation has been taken and lasts as a rule after a single dose 4–10 days. Upon the basis of many experiments, different authors have set the duration of arsenic elimination at a much longer time, for they have succeeded in detecting it in urine 80 and even 90 days after poisoning. Therefore in suspected arsenic poisoning the urine of the patient first of all should be tested for this poison! In arsenic poisoning the quantity of urine is usually much diminished and almost always contains albumin and blood-corpuscles.

Distribution and Deposition of Arsenic in the Human Body

In a series of investigations published in 1880 Ludwig[1] furnished positive proof that the liver is the principal depository of arsenic administered by the mouth. Ludwig examined different parts removed at post-mortems from cadavers of persons who had died of arsenical poisoning. He also determined the quantity of arsenic in different parts of cadavers of dogs in which acute and chronic arsenical poisoning had been produced. Ludwig undertook this investigation mainly because of a research carried out in 1876 in Gautier's laboratory by Scolobusoff. The latter had reached the conclusion that in acute poisoning arsenic first reaches the brain and then quickly passes into the spinal cord, and also that in chronic poisoning most of the arsenic remains in the brain and spinal cord; whereas the liver and muscles contain much smaller quantities of arsenic. In one case Scolobusoff found 40 times more arsenic in the brain and spinal cord than in the same weight of muscle and 3 times more than in the same weight of liver. On the contrary, according to Ludwig, both in acute and chronic arsenical poisoning only extremely small quantities of arsenic are found in the brain, whereas a large quantity of arsenic can be detected both in the liver and kidneys, particularly in the latter organ in acute cases. In chronic poisoning, if administration of the poison is stopped, arsenic is retained longest in the liver. Ludwig found arsenic in abundance in the liver of a dog 40 days after the last dose, whereas the brain, bones and muscle contained no arsenic.

[1] E. Ludwig: Distribution of Arsenic in the Animal Organism after Administration of Arsenious Acid. Med. Jahrb. Wien. 1880, 4H.

Contrary to Armand Gautier, who claims to have found arsenic occurring normally in the human thyroid gland, Hödlmoser[1] found this organ as well as the liver of individuals, who had died from various diseases, free from arsenic without exception. Hödlmoser also obtained entirely negative results from experiments with thyroid glands of sheep and swine, and from the nuclein prepared from these by peptic digestion. Arsenic could not once be found in minute traces in the thyroid glands examined.

In a forensic-chemical examination the contents of the stomach and intestines should claim attention first of all, for sometimes poison that has not been absorbed may still be found there. Among the organs taking up arsenic, the liver should be considered first, for in arsenical poisoning usually the poison is deposited there in largest quantity. Other organs that should be examined are the spleen, heart, kidneys and lungs. In most instances the brain contains merely traces of arsenic, contrary to the claim of Scolobusoff and Gautier that in both acute and chronic poisoning the brain and spinal cord contain the largest deposits of arsenic. The ectodermal organs also appear to be concerned in the fixation and elimination of arsenic, for this poison may accumulate in hair, skin and nails. In 4 cases of severe salvarsan poisoning with fatal termination, the author determined the quantity of arsenic in organs and urine of these individuals, men varying in age from 30–38 years, and in every instance found most of the arsenic in the liver and only traces in the kidneys and urine. In 3 cases the brain was found arsenic-free, although in each only half of the organ was examined; in the brain of the fourth individual minute quantities of arsenic were detected.

Johnson and Chittenden[2] also found in arsenical poisonings most of the poison in the liver. In the case of a woman poisoned by arsenic trioxide they found the following quantities of metallic arsenic:

Organ	Metallic Arsenic
Stomach and spleen	0.0104 per cent.
Kidneys	0.0083 per cent.
Liver	0.0081 per cent.
Lungs and heart	0.0033 per cent.
Uterus and intestines	0.0026 per cent
Lungs and fluid from the thorax	0.0014 per cent.
Bladder	noticeable traces
Brain	unweighable traces

The quantity of arsenic was also determined in bones and muscles. It varied from 0.00016–0.00086 per cent. in the individual parts of the arm and leg.

Similar results were obtained from the cadaver of a dog fed for 8 days with arsenic and examined 24 hours after the last, non-lethal dose: Intestines 0.002; liver 0.001; kidneys 0.0005; muscles 0.0002; urine taken from the bladder 0.0002 per cent. of arsenic. The blood contained noticeable traces of arsenic, whereas the brain was nearly arsenic-free.

[1] C. Hödlmoser: Do Certain Organs of the Human Body Contain Arsenic Physiologically? Zeitschr. f. physiol. Chem. 33 (1901), 328.

[2] S. W. Johnson and R. H. Chittenden: Distribution of Arsenic in the Human Body in a Case of Arsenic Poisoning. Amer. Chem. Journ. 2 (1881), 332.

Recently Gascard[1] published two cases of arsenical poisoning, one running a slow and the other a rapid course. In one case (I) a woman died 22 hours after eating a cake containing about 1 gram of arsenic trioxide. Arsenic was determined in organs that had not begun to putrefy. In the other case (II) a man took about 0.4 gram of arsenic in soup. Although he received immediate medical attention and had his stomach washed out, death ensued after an illness of 9 days. The following quantities of arsenic, calculated in each case for 1 kilogram of organ, were found:

Organ	Case I	Case II
Stomach	224 mg. As_2O_3	2 mg. As_2O_3
Small intestine	223 mg. As_2O_3	6 mg. As_2O_3
Liver	44 mg. As_2O_3	8 mg. As_2O_3
Kidneys	14 mg. As_2O_3	7 mg. As_2O_3
Brain	0.1 mg. As_2O_3	5 mg. As_2O_3

A noticeable fact about Case II is that the quantity of arsenic in the brain is about 50 times more than in Case I. This may be characteristic of arsenical poisoning running a slow course and is probably closely related to disturbances of the nervous system, frequently observed in such poisonings.

Ekeley[2] determined the quantity of arsenic in organs and other parts of the body of a man who had died from arsenical poisoning. The cadaver had lain in the ground 6 weeks before the examinations were made. The largest quantities of arsenic were found in the stomach, liver and kidneys. On the other hand, in percentages the kidneys and walls of the stomach contained most arsenic, next the liver and walls of the intestines, the heart somewhat less, and the brain and spinal cord only a trace. Muscular tissue, especially the thigh and toes contained quite considerable quantities of arsenic. Since arsenic was also found in these, Ekeley assumes that muscular tissues carry on distribution of arsenic through the body.

Heffter[3] has made the following statements with regard to deposition of arsenic in hair: When inorganic or organic arsenical compounds have been taken once or several times, arsenic is stored in the hair. Arsenic enters only into living hair. Dead hair on the contrary will remove nothing from an arsenical solution. Arsenic is firmly held in hair in a form of combination insoluble in water and alcohol. In such cases the amount of arsenic in hair varies from 1 to 5: 100,000. In point of time, passage of arsenic into hair takes place later than deposition of arsenic in abdominal organs. Consequently in acute poisonings running a rapid course arsenic is found in abdominal organs but not in hair. After chronic administration of arsenical preparations medicinally, arsenic has been found in hair at least 14 days after medication began. Disappearance of arsenic from hair requires a very long time so that it may be detected years after, even when

[1] A. Gascard: Two Cases of Poisoning by Arsenic Trioxide, one Acute and the other prolonged. Journ. Pharm. Chim. (7) 7 (1913), 329.

[2] J. B. Ekeley: Distribution in the Human Body of Arsenic administered before Death. Journ. Amer. Chem. Soc. 35 (1913), 483.

[3] A. Heffter: Deposition of Arsenic in Hair. Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw. (3) 49 (1915), 194.

arsenic has long since left the liver and bones. Detection of arsenic in liver and kidneys and its absence at the same time in hair indicate acute arsenical poisoning. If, however, arsenic is found in hair and other parts of the body contain none, the supposition is in some cases that the poison was taken many years ago. On account of the usually high arsenic-content of hair, 5 grams are sufficient for an examination.

Normal Occurrence of Arsenic (?), Copper and Zinc in the Human Organism

In case of many metals introduced into the human body, the liver is the most important organ concerned in taking them up and frequently contains small quantities of heavy metals that have usually found their way into the body with food. Copper, at least in traces, is frequently encountered in the liver during toxicological examinations. Lehmann[1] has given the following figures for the quantity of copper in 1 kilogram of liver: Man 2.5–5 mg.; beef-cattle 22–51 mg.; calf up to 48 mg.; sheep up to 18 mg.

Van Itallie and van Eck[2] found that 5 mg. of copper in 1 kilogram of liver, the maximum quantity given by Lehmann, was almost uniformly exceeded in the case of Dutch livers. They found 3.2–19 mg. of copper in the same quantity of liver. Copper is apparently eliminated in the liver even during foetal life and in larger quantity than in succeeding periods of life. At least these investigators found 26.1 mg. of copper in the liver of a still-born child and 30 mg. in that of a child several hours old, referred to 1 kilogram of the organ.

According to van Itallie and van Eck (loc. cit.), zinc is said to occur almost uniformly in the human liver. They found quite considerable quantities, that is, 17–87 mg. of zinc in 1 kilogram of liver. In examining livers for metallic poisons, the author has repeatedly found traces of copper but zinc only very rarely. In case such traces of copper or zinc are found in the liver or in any other organ, the greatest care must be observed in drawing conclusions from the results. Without further investigation in a case of this kind the conclusion should not be drawn that poisoning from one or the other metal has taken place. Raoult and Breton[3] also determined copper and zinc in human livers and found quantities, varying between 3–15 mg. of copper and 10–76 mg. of zinc in 1 kilogram of the organ. Ogier[4] before that had observed: "On trouve du zinc dans les organes souvent, mais pas toujours, de même qu'on y trouve parfois du cuivre. Mais il s'agit toujours de très petites doses."[5]

According to van Itallie and van Eck, on the other hand arsenic, contrary to the statement of Gautier, is not a normal constituent of human liver. Gautier[6]

[1] Lehmann: Occurrence and Estimation of Copper in Organic Substances. Arch. f. Hyg. 24, 1. 18 (1895), 73.

[2] L. van Itallie and J. J. van Eck: Occurrence of Metals in the Liver. Pharm. Weekblad. 49, 1157; Arch. d. Pharm. 251 (1913), 50.

[3] Raoult and Breton: Natural Occurrence of Copper and Zinc in the Human Body. Compt. rend. de l'Acad. des Sciences 85 (1877), 40.

[4] Traité de chimie toxicologique, page 351.

[5] "Zinc is often found in organs but not always, just as copper is sometimes found. But it is always a matter of very small quantities."

[6] A. Gautier: Report concerning the International Congress of Pharmacy in Paris in 1900; also Pharmazeutische Zentralhalle 41,502.

claims to have found traces of arsenic in the thymus gland and brain of all carnivorous and herbivorous animals as well as of man, and even 0.001 gram in 0.7 gram of thyroid gland. Moreover Schlagdenhauffen and Pagel found arsenic in the thymus glands of various young animals and in the testicles and ovaries of man, oxen, cows and horses. Van Itallie and van Eck examined the livers of 25 persons of very different age and different sex for copper and zinc and found the values given above for these metals. They were able only once to detect arsenic positively in a human liver. The deceased, however, previous to death had taken arsenic medicinally! Obviously in forensic-chemical investigations the almost normal occurrence of copper and zinc in traces in the liver must be taken into consideration. These two investigators were unable to find any relation between sex, age, occupation, residence and the quantities of copper and zinc observed.

ANTIMONY

Experiments upon animals, into the blood of which, or subcutaneously, sodium tartar emetic was injected, have shown that the action of antimony is very similar to that of arsenic, the only difference being that poisoning appears more slowly. On the other hand, the action of tartar emetic taken by the mouth is not identical with that of arsenic. It differs from arsenic in being a stronger local irritant. Owing to the strong local irritation of the stomach, even doses that are not lethal produce emesis through reflex action, whereas arsenic produces emesis only in toxic doses and for this reason cannot be employed therapeutically as an emetic. Moreover it is possible to produce pustulous skin eruptions more surely with tartar emetic ointments than with those containing arsenic.

After internal administration, antimony is eliminated in great part before absorption by emesis. A part of the poison may be deposited in an insoluble condition in the liver and possibly also in the bones. Even traces of antimony have also been found in the brain and spinal cord. Pouchet found antimony in the skin, hair, bones and especially in the intestinal tract. Materials to be taken into consideration for chemical examination are: vomitus, mucous membrane of stomach and intestines, stomach and intestinal contents, liver, and also brain, skin, hair and urine.

BARIUM

Of the barium compounds, only barium hydroxide, sulphide and more concentrated solutions of neutral barium salts act as local irritants. On the other hand, all soluble barium salts may produce effects due to absorption. These concern the heart and blood-vessels, gastro-intestinal canal, central nervous system, that is, picrotoxin-like stimulation then paralysis, and muscles. Elimination of barium takes place by way of the salivary glands, intestinal glands, and in small quantity by the urine.

The effects of intoxication are nausea, flow of saliva, retching, emesis, very painful colic, and severe diarrhoea. A noticeable feature is the digitalis-like influence upon the heart and blood-vessels, manifesting itself in slow pulse and strong palpitation of the heart. The blood-pressure is strikingly increased. Additional symptoms are: dizziness, ringing in the ears, deafness, visual disturb-

ances and a feeling of anxiety. Catarrhal affections of the conjunctiva and of the mucous membrane of the respiratory tract, that is, of the nose, have been observed in man (R. Kobert). In one case described in the literature, 10 grams of barium chloride proved to be a lethal dose; in another case 3 grams of the same barium salt proved fatal. The autopsy shows in the mouth swelling of the salivary glands; the stomach and duodenum in particular exhibit inflammatory changes and are very red; the heart, lungs and brain are highly congested.

Experiments upon animals have shown that distribution of barium in fatal poisoning is as follows: the lungs, heart and muscles contain only traces; the liver, kidneys, brain and spinal cord a little more; and the bones most of the barium. Only a small part of the barium is eliminated in the urine. The parts of the cadaver of most importance for chemical examination are: vomitus, stomach-contents, faeces and urine, the latter especially after rather large doses of barium have been taken.

TOXIC ACTION OF HEAVY METALS

Most salts of heavy metals, such as lead, copper, mercury, silver, uranium, bismuth and zinc, produce precipitates with protein substances, forming metal-albuminates. In combining with oxides of these metals, proteins behave like acids. If the metal-albuminates first formed are insoluble, or only slightly soluble in the body-fluids, they are non-toxic or only slightly toxic. But a soluble albuminate is transported throughout the organism and exerts a toxic action. Every cell in contact with the dissolved metal may be poisoned. Mercury albuminate is an example of the latter class of metal-albuminates. On account of its solubility in sodium chloride and protein solutions, it acts as a powerful poison. Copper albuminate on the other hand is not appreciably soluble in solutions of sodium chloride, hydrochloric acid or proteins. Not entering the circulation, it is as good as non-toxic. Lead and silver albuminates are like copper albuminate as regards solubility in the solvents mentioned. But if a heavy metal, forming a difficultly soluble albuminate, finds its way into the organism in organic combination, that is, in a non-ionizable form, for example, copper in union with tartaric acid, so that it cannot be precipitated by proteins, then it is as poisonous, or nearly as poisonous, as mercury in corrosive sublimate. Administration intravenously of 20 mg. of such copper causes the death of an adult rabbit.

Consequently precipitation takes place wherever the salt of a heavy metal comes in contact with proteins. The term corrosion is applied to such an occurrence. There is always present the metallic oxide, protein and acid originally combined with the metal. As a rule the acid is loosely held by the precipitate and is washed away by the circulating blood. The corrosive action of salts of heavy metals is due both to the union of metallic oxide with protein, living protein being changed to dead metal-albuminate, and to the caustic action of free acid. Therefore the intensity of the action of the salt of the heavy metal depends upon the nature of the metal-albuminate. The degree of solubility in the body fluids is especially important, as well as the quantity and strength of free acid. Salts of heavy metals not only may affect the place of application, but they may give rise to serious changes where eliminated, as in intestines or

kidneys. Before elimination they may also seriously harm parenchymatous organs like the liver, as well as circulatory organs. Finally salts of heavy metals have an important action upon the blood. R. Kobert and collaborators have found that white as well as red blood-corpuscles may combine with metals and act as antidotes. Kobert has shown that the substance of red blood-corpuscles is capable of taking up a considerable quantity of a heavy metal. A chemical compound (metal-haemoglobin) is formed and the oxyhaemoglobin spectrum is not changed. Thus lead speedily impairs vitality of red blood-corpuscles. Consequently red blood-corpuscles are killed in large quantity in lead poisoning.

LEAD

Lead is eliminated in urine and faeces. Elimination by faeces always exceeds that by urine, even when lead has not been taken by the mouth. Mann,[1] for example, in the case of two patients was never able to find more than 0.6 mg. of lead in urine collected during 24 hours, whereas faeces during the same period contained 2–3 mgs. of lead. In lead poisoning the metal has been found in saliva, bile and blood, both in red and white blood-corpuscles. In animals relatively most of the lead has been found in the kidneys, after which come bones, liver, testes and finally brain and blood. In experiments with sheep, Ulenberger and Hofmeister obtained the following results: Kidneys 0.44–0.47; liver 0.3–0.6; pancreas 0.54; salivary glands 0.42; bile 0.11–0.40; bones 0.32; faeces 0.22; spleen 0.14; blood 0.05–0.12; urine 0.06–0.08 gram per 1000 grams of each material examined.

Lead is eliminated especially by the bile and in acute poisoning this secretion may contain more lead than any of the other organs or secretions. Oliver[2] obtained the following results from human material: Liver 41.6; spleen 39.0; large brain 21.6; small brain 8.6; kidneys 10.0; and heart 0.5 mg. per 1000 grams of each material examined. Elimination of lead by the urine in the case of man is not always uniform. Urine is free from lead for a long time and later without further administration again contains the metal.

Elimination of lead after administration proceeds very slowly by the urine, intestinal glands, skin, saliva and possibly the milk. During continuous administration of small doses of lead salts, therefore, cumulative action takes place. Brouardel considers even a daily dose of only 1 mg. of lead sufficient to produce chronic plumbism in man. If antidotes are not used, the poison in part may be retained in the organism for many months. The metal is abundantly eliminated through the kidneys only while the body is overloaded with lead. Elimination proceeds later chiefly through the intestines. But elimination may cease almost entirely for months notwithstanding the severest symptoms of intoxication. Of all metals none causes such severe injury to the kidneys as lead, for it is capable of producing serious impairment of this organ. Even small quantities of lead in passing through the kidneys bring about lasting illness in the secreting parenchyma of this organ, laying it waste and ultimately causing true cirrhosis of the kidneys (Kobert). In chronic lead poisoning in rabbits Oppen-

[1] Zeitschr. f. physiol. Chemie 6 (1882), 6.

[2] The Lancet, March 1891.

heimer found the following quantities: Brain 199.7; hollow bones 118; kidneys 62.7; bone-marrow 57.7; liver 48; muscles 33.3; and blood 4.9 mg. of lead per 100 grams of each dry material examined. Therefore the brain sometimes may contain quite an abundance of lead! A human brain was also found to contain over 100 mg. of lead in 100 grams of dry material. Blyth isolated relatively more lead from the small brain than from the large brain of a man who had died from Epilepsia saturnina, obtaining 117 mg. of lead sulphate from the entire organ. In another case Blyth obtained 106 mg. of lead sulphate from the entire brain. In one instance the lungs were also found to contain quite a quantity of lead. As a rule lead is so firmly deposited in these organs that the metal can be detected years after in persons formerly poisoned with lead. Lead can also be detected for a long time in the cadaver. In the examination of an exhumed cadaver special consideration should be given to the fact that lead may reach the cadaver later from ornaments or pigments of the coffin containing this metal. Moreover it should be borne in mind that lead is deposited in the brain, spinal cord, bones, bone-marrow, liver, kidneys, intestines and muscles. Consequently in such examinations these parts of the cadaver should first of all be tested for lead.

CHROMIUM

Chromic acid and all soluble chromates and dichromates act as strong poisons. Alkaline chromates are absorbed by the mucous membranes, producing severe, acute poisoning. The poison causes intense pain in the stomach and intestines, collapse and kidney derangement that may terminate fatally in a few hours. Other symptoms are nausea, vomiting of yellow matter that is later tinged with blood, diarrhoea and even bloody stools, intense thirst, emaciation, great anxiety, severe pain in the abdomen, small, feeble and quickened pulse—"the cholera picture" (Kunkel, Toxikologie). Statements with regard to the quantity of an alkaline chromate, capable of producing acute poisoning, agree fairly well. Even a few decigrams (0.2 gram) may cause sometimes dangerous symptoms. Chromic acid is eliminated mainly by the urine and partly by the intestines. Elimination takes place rapidly and the body is soon free of the poison. Four days after administration of quite large quantities of a chromate, the urine and faeces are said to contain only traces of this metal. Compounds containing chromium as a positive ion, that is, chromic salts, are about 100 times less toxic than the chromates.

COPPER

Only a small amount of copper, varying with different compounds, is absorbed by the intestines and carried into circulation. Sodium cupric tartrate and copper salts of fatty acids are absorbed most easily. Copper poisoning rarely occurs from introducing a copper compound into the stomach. Copper compounds in large amounts act locally as corrosives, causing severe gastric pains. Vomiting and sense of taste make it impossible to take much of a copper compound. Foods containing copper are unpalatable. Sense of taste as well as after-taste prevent one from swallowing such food in any quantity. Food containing 0.5 gram of copper per kilogram has a marked taste. Irresistible nausea, steadily increasing,

soon makes it impossible to take more of the food containing copper. Elimination of copper by the urine is very slight. Copper absorbed from the intestines is arrested by the liver where it accumulates. Traces of copper have frequently been found in the human liver. In toxicological examinations the author has repeatedly found weighable quantities of copper in livers of adults who had not taken copper salts beforehand, except possibly for suicidal purposes. The liver is the most important organ for detection of copper, next to which come bile, kidneys and gastro-intestinal mucosa. Copper is said to be in the liver as a nuclein compound. In the case of blood, copper is located not in the serum but in the corpuscles.

MERCURY

Mercury is easily absorbed from all mucous membranes, wounds and, in the case of a corrosive mercury compound, such as corrosive sublimate, also from the intact skin. Corrosive action is partly due to precipitation of protein and partly also to the fact that a mercury salt as a result of hydrolysis gives off acid which then exerts corrosive action. Mercury albuminate formed (see Toxic Action of Heavy Metals, page 270) enters the circulation, for it is dissolved by alkaline chlorides in the blood. Once in the circulation, mercury is distributed in the kidneys, liver, spleen, intestines, heart, muscles, lungs, brain salivary glands, bile and bones.

Distribution of mercury in the body is said always to be the same, no matter what be the method of administration. It is immaterial whether it is introduced by the mouth, hypodermically, or from an abrasion. Elimination of mercury takes place through the saliva, sweat, bile, gastro-intestinal mucosa, milk and especially through the urine. Elimination in the saliva seems to be constant, since mercury can always be detected in saliva during use of mercurials in lues. A relatively large quantity of this metal is said to be eliminated in sweat. Opinions differ as to the relative quantities eliminated by urine and intestines. Usually elimination by the intestines exceeds that by the kidneys. Recent experiments seem to show that mercury is eliminated in urine regularly and in slowly increasing quantity and slowly diminishes. Elimination of mercury ceases after 6–9 months and even later. In a most favorable case the total quantity of mercury eliminated in urine amounts to about 50 per cent. of the total quantity taken but frequently is much less. In mercurial poisoning the kidneys of all organs always contain most mercury and even for weeks. Then follow liver, spleen, bile and intestinal mucosa. In toxicological examinations urine should also be examined, although in acute poisoning it always contains only a fraction of a milligram of mercury in a liter. In severe mercurial poisoning the metal may be said to occur in all organs and secretions.

Soon after administration the action of mercury is apparent. Even after half an hour death may occur or not until days or weeks have passed. Mercury circulating in the blood as mercury albuminate produces inflammation of the mucous lining of the mouth (stomatitis), flow of saliva and severe irritation of the intestines. The latter may give rise to ulcers in the entire colon even when not a trace of mercury has been introduced primarily into the gastro-intestinal tract. Mercurials have a very decided action upon the mouth. It is immaterial whether the preparations are rubbed in, injected, or administered by the mouth. The effects

are flow of saliva, blackening, swelling, and formation of ulcers on gums and tongue. Probably this mercurial stomatitis is connected with elimination of mercury through the saliva and mucous lining of the mouth. Poor teeth actually favor it. Blackening at the margins of the gums is due to formation of mercury sulphide. In severe cases necrosis of the jaws may occur. In short, acute mercurial poisoning manifests itself in inflammation and swelling of the mouth, pains along the digestive tract, vomiting of bloody, mucous masses, bloody stools, and frequently complete anuria. The findings at autopsy are loosened and discolored gums, ulcers, severe inflammation of stomach and intestinal tract, and possibly also perforation. Frequently a slate-gray coloration of the entire mouth, oesophagus, and gastric mucosa is observed. There may also be bleeding and ulcers in the gastric mucosa. Not infrequently acute inflammation and fatty degeneration or calcification of the epithelium of the kidneys are found.

SILVER

In acute poisoning in man the local, corrosive action of silver nitrate in the form of lunar caustic is almost all that has to be considered. With the tissues of the body it forms scabs of silver albuminate that are dry, white and do not extend very deep. These corrosions of the outer skin, resulting from reduction, immediately turn black, especially in the light. All human tissues possess reducing properties but in different degree. The action of living protoplasm in reducing silver solutions is stronger than that of decaying or dead protoplasm.

Urine in acute silver poisoning usually does not contain this metal, whereas it may be found in the intestinal contents even after subcutaneous injection of silver. Part of the soluble silver preparation injected may be seen under the microscope within a few days as brown or deep black points in various organs, such as liver, spleen, kidneys, pancreas, bone-marrow and intestinal mucosa (acute argyria). In chronic argyria the finger nails are first colored dark and frequently also the margin of the gums. This recalls the appearance of the gums in lead and mercurial poisoning but the color is violet, whereas in the case of lead it is more grayish. Later grayish black spots develop also in other parts of the skin and these finally may produce a uniformly dark coloration of the skin where it is exposed to light. Silver salts form such exceedingly stable compounds with protein that there is no way of restoring the natural appearance of skin once discolored by silver. The affinity of protein for silver probably exceeds that of the halogens for this metal. Only a trace of silver reaching the intestines is absorbed. After silver medication, stools are black from silver sulphide. Silver has frequently been determined in organs of persons who have had argyria. In one case the liver contained 0.047 and the kidneys 0.061 per cent. of silver, calculated for the dry organs; and in a second case the liver and kidneys in fresh condition contained respectively 0.018 and 0.03 per cent. of silver.

From every indication the lethal dose of silver preparations is quite high, at least a suicide recovered after having taken 30 grams of silver nitrate.

After administration of silver nitrate there appears as with all corrosives, provided the poison is not immediately expelled by emesis, gastro-intestinal catarrh with burning, severe pain in the abdomen, and vomiting of white masses that turn dark in light. The mucous lining of the mouth is whitish. Dizziness, unconsciousness, paresis, and complete paralysis of individual members of the

body may occur. Death may result from asphyxiating convulsions or from oedema of the lungs (Kobert).

In the cases of poisoning recorded in the literature the autopsy revealed white coloration and superficial scabbing of the mucous membrane of the throat, inflammation and corrosion of the gastro-intestinal tract which here and there was whitish. Dark coloration of the internal organs as well as of the skin was lacking.

URANIUM

Experiments by R. Kobert have shown that uranium, administered subcutaneously or intravenously, is the most toxic of all metals. Uranyl acetate is an excellent precipitant of proteins and other uranyl salts are probably hardly less inferior in this respect. Consequently internal administration of concentrated solutions of uranyl salts destroys the mucous surfaces they touch, for example, that of the stomach, changing the living stomach-wall into dead uranyl-albuminate. Therefore uranyl salts must be classed among the powerful corrosive poisons. In addition to acting as local corrosives, uranium salts resemble hydrocyanic acid in partially arresting internal oxidation in organs and occasioning severest disturbances of metabolism.

Uranium trioxide (UO_3), administered subcutaneously even as a non-corrosive double salt, acts fatally upon cats, dogs and rabbits in doses of 0.5–2 mg. per kilogram of body weight. A very dilute, non-corrosive double salt administered internally in small doses is for the most part unabsorbed. Yet it produces very severe poisoning. Uranyl acetate taken internally produces inflammation of the gastro-intestinal tract. Uranium salts in minimal quantities, such as do not produce gross anatomical changes, give rise to glycosuria and larger doses to albuminuria, cylinduria and blood in the urine. Gastro-intestinal inflammation also appears after subcutaneous administration of non-corrosive sodium uranyl tartrate which is evidence that the poison is eliminated by the gastro-intestinal mucosa. Consequently only traces of uranium are found in the urine. In the chemical detection of uranium the contents of stomach and intestines should receive first consideration.

BISMUTH

This metal becomes quite toxic when it reaches the blood. Bismuth solutions, prepared by dissolving bismuthous hydroxide in tartaric or citric acid and then neutralizing with sodium or ammonium hydroxide solution, have been repeatedly administered to animals subcutaneously and intravenously. The smallest lethal dose of these double bismuth salts, injected subcutaneously, was found to be but 6 mg. per kilogram of body weight for a dog or cat and 24 mg. for a rabbit. Bismuth salts insoluble in water produce entirely different results when administered internally. Bismuth subnitrate and similar salts dissolve very slightly in the highly diluted hydrochloric acid of the gastric juice and consequently very little bismuth is conveyed to the blood. Most of the bismuth taken by the mouth reaches the intestines where it is not absorbed but converted into bismuthous sulphide by hydrogen sulphide always present there. Absorbed bismuth is eliminated by the saliva, bile, urine, mucous lining of the mouth, stomach, small and

large intestine and also milk. If an animal is poisoned by bismuth, the metal can be detected in urine, bile, liver, kidneys, spleen, walls of the intestines, as well as in the bones. Different observers have found the metal in especially large quantity in milk but very little in the kidneys and liver.

Subnitrate of bismuth has given rise to poisonings but is less likely to produce such a result after internal than after external use. Application of 10 per cent. bismuth ointment for a burn proved fatal. The gums had a blue-black color and the parenchyma of the kidneys degenerated. Changes in the mouth are similar to those appearing in mercurial poisoning: flow of saliva, swelling of the gums, tongue, salivary glands, and mucous membrane of the cheeks and throat, loosening of the teeth, blackening of the margins of the gums, and ulceration of the mouth. After a cure has been effected, the mucous surfaces attacked remain blackened. Moreover there is intestinal catarrh and nephritis like that caused by corrosive sublimate.

ZINC

Without question zinc salts upon reaching the intestinal tract are absorbed only in small quantities. As yet there is no satisfactory explanation of the fate of absorbed zinc. In zinc poisonings rather large quantities of this metal have repeatedly been found in the liver and bile. This may mean that zinc is arrested by the liver and eliminated in the bile. In an experiment made by Lehmann,[1] a dog was fed for some time with zinc carbonate and killed after 335 days. The following organs, arranged according to the quantity of metal in each, contained zinc: liver, bile, large intestine, thyroid gland, spleen, pancreas, urine, kidneys, bladder, muscle, brain, lymphatic glands, stomach, small intestine, lungs and blood. A fact deserving mention is that quite appreciable quantities of zinc may be acquired from foods. All acids dissolve metallic zinc very freely. Even water containing carbon dioxide is a solvent. Consequently it may be in drinking water from galvanized pipes. All kinds of food and drink, kept in zinc vessels or those coated with zinc, may contain this metal. Moreover plants grown upon soil containing zinc take up this metal. Zinc has also been repeatedly found in parts of human cadavers under circumstances precluding all possibility of poisoning by this metal. Even considerable quantities of zinc have been found in the human liver.

Zinc chloride and sulphate belong to the corrosive poisons, the former in particular having very strong corrosive properties and the latter acting as a powerful emetic. As a result of formation of zinc albuminates, these local corrosive effects produce a firm dry scab. A distinction should be drawn between these corrosive effects and general effects that may manifest themselves in paralysis of the central nervous system soon followed by paralysis of the vascular system, heart and muscles, as demonstrated by experiments where zinc compounds have been injected direct into the blood. Zinc resembles almost all heavy metals in combining with the haemoglobin of the blood. Not only is zinc eliminated by urine but it is said also to be found to some extent in bile and milk. The smallest lethal dose observed after internal administration amounted to 6 grams of zinc chloride and 7.6 grams of zinc sulphate. Occasionally even larger quantities of these two zinc salts have been tolerated.

[1] Archiv f. Hygiene 28 (1896), 219.

Poisoning from zinc sulphate resembles acute poisoning from tartar emetic, for it soon produces severe emesis. It differs, however, from the latter in creating an intensely astringent, metallic taste and in giving the mucous lining of the mouth a white, wrinkled appearance. The stomach and intestines may be highly inflamed. Notwithstanding the fact that the stomach was washed out, the tendency to emesis persisted in several poisonings from 50 per cent. zinc chloride solution that terminated fatally. Very severe vomiting, often of blood, occurred. In a case observed by v. Jaksch, where a drinking glass full of 68 per cent. zinc chloride solution containing hydrochloric acid was taken, severe nephritis, fever, and death on the sixteenth day from septic infection ensued. In earlier stages haematoporphyrinuria and glycosuria were in evidence.

TIN

Cases of tin poisoning thus far observed resemble those of copper and zinc. What knowledge there is with regard to the toxic action of absorbed tin has been gained from experiments upon animals. These experiments show that small quantities of tin are absorbed and eliminated in the urine, when ordinary tin compounds are brought into the stomach. But thus far distinct symptoms of poisoning by such quantities of the metal have not been confirmed. (Kunkel, Toxikologie.)

White[1] failed to produce poisoning by introducing a tin compound into a dog's stomach. The animal received sodium stannous tartrate in increasing doses for 22 days, the daily amount being 0.02–0.06 gram. Yet the animal absorbed tin. In the urine, during an experiment lasting 8 days, White found 0.02 gram of tin. But the tin salt mentioned, introduced directly into the circulation of the animal, was quite toxic in action. Stannous chloride, administered for a very long time to a dog, produced symptoms of poisoning. The urine in this case contained small quantities of tin. Kunkel (Toxikologie) states that tin has a very slight poisonous action. Apparently it is eliminated very rapidly by the kidneys. Quite probably this prevents accumulation of the metal in the body and consequent poisoning. The fact that White did not observe toxic symptoms, after feeding a dog for 22 days with relatively large quantities of easily absorbable sodium stannous tartrate, and that Ungar and Bodländer[2] failed to produce derangements with the same compound, until it had been administered for a year, prove that tin is quite free from toxic properties. Hence tin vessels may be used and preserved articles of food containing tin have practically no deleterious action upon health.

Lehmann has come to the conclusion from his experiments that rather large quantities of tin salts may be taken without occasioning more than a milder form of digestive disturbance. Yet these same quantities taken more frequently may also cause disturbance of the nervous system. Quantities of tin, however, necessary to produce the latter effect are far greater than those found in preserves. Consequently ordinary preserves that are not acid, or not strongly so, may be regarded as harmless as far as the quantity of tin they contain is concerned. Lehmann therefore considers it desirable not to put up strongly acid

[1] Archiv f. experimentelle Pathologie u. Pharmakologie 13, 53.

[2] Zeitschrift f. Hygiene 2, 241.

products, particularly those containing tartaric or malic acid, in tin boxes but in glass, porcelain or wooden containers.

SYNOPSIS OF GROUP I

Systematic Procedure in Chemical Examination for Poisons

Before distillation, test a rather small portion of material for phosphorus by **Scherer's test** and a second portion for hydrocyanic acid by **Schönbein's test.**

If this preliminary test for phosphorus is positive, distil in the **Mitscherlich apparatus,** otherwise conduct distillation with Liebig condenser in the usual position. In both cases material should be comminuted as finely as possible, stirred with water to a thin mass, acidified with tartaric acid, and distilled in a capacious glass flask either over free flame or from oil-bath. In many cases it is advisable during distillation to pass through the material carbon dioxide washed with water and to warm only upon the water-bath. This is the method used for detection and quantitative determination of hydrocyanic acid and phosphorus in cadaveric material. It is an advantage to collect the distillate in 2–3 fractions. Test the first 5–10 cc. for hydrocyanic acid, chloroform, alcohol, methyl alcohol and possibly for iodoform and nitrobenzene. The other fractions, containing possibly less volatile substances, are used to test for carbolic acid and other phenols volatile with steam, as well as for chloral hydrate, aniline, formaldehyde, phosphorus and carbon disulphide.

Phosphorus.—See page 1. **Phosphorescence in Mitscherlich apparatus** during distillation in a dark room. Distillate, evaporated with strong chlorine or bromine water, or fuming nitric acid, gives phosphoric acid, recognized by **test with ammonium molybdate** or **magnesia mixture.** As an alternative procedure, test original material for phosphorus, as well as phosphorous acid, by **Blondlot-Dusart method.**

Hydrocyanic Acid.—See page 27. **Preliminary test:** blue color on paper strip moistened first with guaiac tincture and then with a little copper sulphate solution. **Prussian blue test. Sulphocyanate test. Silver nitrate test:** white, flocculent silver cyanide soluble in ammonia.

Carbolic Acid and Cresols.—See pages 43 and 52. **Odor.** Red color with **Millon's reagent** upon warming. Yellowish white, crystalline precipitate of tribromo-phenyl hypobromite with **bromine**

water. Violet color with ferric chloride, passing into yellow upon acidification with mineral acids. **Examine urine,** determining especially sulphate and ethereal sulphuric acid. The latter is greatly increased in phenol poisoning.

Thymol.—See page 54. **Odor** of thyme. Distillate frequently contains oil-drops. Extract with ether and evaporate ether. Test oily residue for thymol: concentrated sulphuric acid. **Piria's, Eykmann's, Liebermann's** and **Lustgarten's tests.** No red color with **Millon's reagent. Bromine Water** gives only milkiness but no crystalline precipitate (distinction from carbolic acid and other volatile phenols).

Naphthols.—See page 57. Extract distillate with ether and evaporate solvent. Test residue for naphthol: **Chlorine water. Ferric chloride. Piria's test. Chloral hydrate test. Formaldehyde test. Lustgarten's test. Millon's test. Diazonium test.** Differentiate α- from β-naphthol by **Jorissen's** and **reverse Molisch's test.**

Chloroform.—See page 59. **Odor.** Repulsive odor of **phenyl-isocyanide** upon heating with little aniline and potassium hydroxide solution. Red color upon heating with resorcinol and potassium hydroxide solution. Blue color upon gentle heating with α-naphthol and potassium hydroxide solution. Reduction upon warming distillate containing chloroform with ammoniacal silver nitrate solution and Fehling's solution.

Chloral Hydrate.—See page 64. Gives chloroform reactions. Also aldehyde test with **Nessler's reagent:** Brick-red precipitate, becoming after some time yellow or dirty green. Gives chloroform and magnesium formate when heated with magnesium oxide and water. Test for formic acid by warming with silver nitrate or mercuric chloride solution. Occurs in urine, after administration of chloral hydrate, as urochloralic acid.

Butyl Chloral Hydrate.—See page 67. Reacts like chloral hydrate with Nessler's reagent. But differs from it in not giving chloroform with caustic alkalies, nor the phenyl-isocyanide test.

Iodoform.—See page 68. **Odor.** Distillate milky, whitish. Ether extract of distillate leaves crystals upon evaporation. Distillate containing iodoform gives chloroform reactions, especially well **Lustgarten's test.**

Aniline.—See page 71. Violet color with calcium hypochlorite solution. Repulsive odor of **phenyl-isocyanide,** heated with 1–2

drops of chloroform and potassium hydroxide solution. Flesh-colored precipitate with **bromine water.** Red color on warming with **Millon's reagent** and evolution of nitrogen. **Chromic acid test.**

Nitrobenzene.—See page 69. Oil drops in distillate with yellowish color and characteristic odor. Separated in separating funnel, or better extracted with a little ether. Evaporation of ether leaves nitrobenzene. Reduce by shaking with tin or zinc and little concentrated hydrochloric acid, make alkalkine with potassiumh ydroxide solution, extract with ether, and test residue from ether for aniline.

Alcohol.—See page 73. Gives **iodoform** upon gentle warming with iodine and sodium hydroxide solution. Odor of **ethyl benzoate** when thoroughly shaken with benzoyl chloride and sodium hydroxide solution. Green color and odor of **acetic aldehyde** when warmed with trace of potassium dichromate and hydrochloric acid. Odor of **ethyl acetate** when warmed with little sodium acetate and concentrated sulphuric acid.

Acetone.—See page 76. Gives **iodoform** even in cold with iodine and sodium hydroxide solution, more slowly with ammonia. **Legal's test.** Dissolves mercuric oxide when shaken with mercuric chloride and excess of alcoholic potassium hydroxide solution. Therefore black zone when clear filtrate is covered with ammonium sulphide. Gives **indigo** with o-nitro-benzaldehyde and sodium hydroxide solution.

Isoamyl Alcohol.—See page 78. Extract distillate with chloroform and evaporate solvent. Oil drops having characteristic odor. Saturated aqueous solution prepared by shaking with little water at 15–16° becomes milky when warmed to about 50°. **Marquardt's test:** odor of isovaleryl aldehyde, and finally of iso-valerianic acid, after standing for some time with little water, dilute sulphuric acid, and potassium permanganate (1:1000). **Uffelmann's test:** violet, or more of reddish blue color, upon addition of freshly prepared solution of methyl violet having green color from hydrochloric acid.

Formaldehyde.—See page 79. Gives **general aldehyde reactions:** precipitation of metallic silver with ammoniacal silver nitrate; separation of red cuprous oxide when heated with Fehling's solution, and gray metallic mercury with Nessler's solution. Red color with alkaline phloroglucinol solution. **Hehner's test:** violet color when warmed with fresh, unboiled milk and hydrochloric acid containing ferric chloride. **Morphine-sulphuric acid:** solution of morphine

hydrochloride in concentrated sulphuric acid; purple-red color by mixing this solution with a mixture of aqueous formaldehyde solution and concentrated sulphuric acid. **Denigès test:** with fuchsine-sulphurous acid in strong sulphuric acid solution; blue to red-violet color, increasing in intensity with time. **Grosse-Bohle-Fincke test:** same color with fuchsine-sulphurous-hydrochloric acid. **Rimini's test:** blue color passing into red when mixed with aqueous solutions of phenyl-hydrazine, sodium nitro-prusside and strong sodium hydroxide solution. **Schryver's test:** fuchsine-like color when mixed with freshly prepared aqueous solutions of phenyl-hydrazine hydrochloride and potassium ferricyanide, and then with concentrated hydrochloric acid. Conversion by ammonia into **hexamethylene tetramine** and detection of latter with (a) mercuric chloride (b) potassium mercuric iodide solution: crystals.

Methyl Alcohol.—See page 89. **Oxidation** to formaldehyde by glowing copper spiral, potassium permanganate and dilute sulphuric acid, potassium dichromate and sulphuric acid, sodium persulphate and dilute sulphuric acid, or platinum black, and detection of formaldehyde by color reaction. **Autenrieth's test:** shake aqueous solution warmed to about 40° with 10 per cent. sodium hydroxide solution and p-bromo-benzoyl chloride; formation of methyl ester of p-bromo-benzoic acid, appearing in fine crystals from alcohol, methyl alcohol, or acetone, having anise-like odor, and melting at 78°; percentage of bromine may also be determined.

Carbon Disulphide.—See page 100. Black color, or black precipitate (PbS), when heated with little lead acetate and potassium hydroxide solution. Formation of ammonium sulphocyanate by evaporation with concentrated ammonia, detected with little ferric chloride after acidification with dilute hydrochloric acid. Formation of potassium xanthogenate, when shaken with alcoholic potassium hydroxide solution, and detection with copper sulphate solution.

SYNOPSIS OF GROUP II

Examination for Organic Poisons Non-volatile with Steam from Acid Solution but Extracted from Material by Alcohol Containing Tartaric Acid. Stas-Otto Method

Material as finely comminuted as possible acidified with tartaric acid, extracted warm under reflux for 20–30 minutes with twice to three times quantity of pure alcohol with occasional shaking, and

filtered. Filtrate evaporated upon water-bath to thin syrup. Latter stirred with water (100–200 cc.), filtrate again evaporated upon water-bath, and residue, while well-stirred, mixed with quite a large quantity of absolute alcohol, to precipitate proteins and other substances as completely as possible. Filtered alcoholic liquid gives upon evaporation residue that dissolves in 50–60 cc. of water usually giving clear or only slightly milky solution that has yellow to brownish color and acid reaction. Acid solution thoroughly and repeatedly extracted direct with ether (A), then after it has first been rendered alkaline with sodium hydroxide solution (B) then also with ammonia (C), and latter alkaline liquid finally with warm chloroform, or with chloroform containing 10 per cent. alcohol (D). Residues left by distilling or evaporating solvents examined according to directions given from A to D. See pages 108 to 225.

A. **Residue from Ether Extract of Aqueous Tartaric Acid Solution May Contain:**

Picrotoxin.—See page 111. **Very bitter.** Reduces **Fehling's solution** when warmed. **Melzer's test:** red streaks radiating from picrotoxin covered with alcoholic benzaldehyde solution and concentrated sulphuric acid. Concentrated sulphuric acid gives yellow or orange-red color; drop of potassium dichromate solution has brown margin. Mixture of picrotoxin and about three times the quantity of potassium nitrate moistened with trace of concentrated sulphuric acid acquires red color with excess of saturated sodium hydroxide solution.

Colchicin.[1]—See page 115. **Very bitter,** yellowish, amorphous. Few drops of mineral acid render aqueous colchicin **solutions intensely yellow.** Dissolves in concentrated nitric acid with dirty violet color, changing at once to brown-red and finally to yellow. Addition of excess of potassium hydroxide solution imparts orange-yellow or orange-red color to mixture. **Zeisel's test:** Heat to boiling yellow solution of colchicin in concentrated hydrochloric acid for 2–3 minutes in test-tube with 2 drops of ferric chloride solution. Green or more of an olive-green color appears upon cooling, especially after addition of about same volume of water. **Führer's test.**

Picric Acid.—See page 119. **Very bitter,** yellow, usually amorphous from ether solution. In presence of picric acid, material and

[1] Because of its slight solubility in ether, colchicin appears in ether extract of the tartaric acid solution only in small quantity. Most of it is found in the chloroform extract of the solution rendered alkaline with ammonia (see D).

various extracts have more or less intensely **yellow color. Isopurpuric acid test:** Aqueous picric acid solution gently warmed with few drops of saturated potassium cyanide solution becomes red. Similarly aqueous picric acid solution becomes red when warmed with little ammonium sulphide. Aqueous picric acid solution **dyes wool and silk yellow, but not cotton.**

Acetanilide.—See page 122. Faint, burning taste. **Indophenol test:** Acetanilide, boiled down to about 20 drops with few cc. of concentrated hydrochloric acid and treated when cold with aqueous phenol solution and calcium hypochlorite solution added drop by drop, gives when shaken dirty red to violet color, becoming deep blue when covered with ammonia. Acetanilide heated to boiling first with alcoholic potassium hydroxide solution and again after addition of little chloroform gives repulsive odor of **phenyl-iso-cyanide.**

Phenacetine.—See page 125. Tasteless. Gives **indophenol but not phenyl-isocyanide test.** Concentrated nitric acid colors phenacetine yellow and dissolves it with yellow to orange-red color. Heated with dilute nitric acid, gives yellow or orange-yellow solutions. If these solutions are saturated, nitro-phenacetine appears as yellow crystals as solutions cool.

Salicylic Acid.—See page 131. Usually needle-like crystals, having sweetish, acidulous, rather harsh taste. Drop of **ferric chloride** solution colors aqueous solution blue-violet, in greater dilution red-violet. Red color when warmed with **Millon's reagent. Bromine water** in excess produces a yellowish white, crystalline precipitate.

Veronal.—See page 137. **Bitter,** crystallizes and sublimes easily. If solution of ether extract in as little sodium hydroxide solution as possible, sodium carbonate solution, or aqueous ammonia is filtered and filtrate acidified with dilute hydrochloric acid, veronal will crystallize. **Melting-point** (187–188°) of dry crystals, as well as that of a mixture of these with absolutely pure veronal, may be determined. It should be the same in both cases. With **Millon's reagent** saturated, aqueous veronal solution gives white, gelatinous precipitate soluble in large quantity of reagent. When shaken this solution foams like soap solution.

Antipyrine.[1]—See page 141.—Mildly bitter taste. Test aqueous solution of residue for antipyrine: Red color with drop of **ferric**

[1] Most of the antipyrine when present is found in ether extract B of the aqueous alkaline liquid as well as in chloroform extract D.

chloride solution. Green color with 1–2 drops of **fuming nitric acid.** If solution is boiled and another drop of fuming nitric acid added, green color changes to red.

Caffeine.[1]—See page 145. Faintly bitter. Evaporated upon the water-bath with saturated chlorine water, caffeine gives red-brown residue. Moistened with very little ammonia, becomes purple-violet. Good procedure (E. Fischer) to boil substance in test-tube with strong chlorine water, or with hydrochloric acid and trace of potassium chlorate, evaporate to dryness in dish upon water-bath, and moisten residue with ammonia.

B. **Residue from Ether Extract of Aqueous Solution Rendered Alkaline with Sodium Hydroxide Solution May Contain:**

Coniine.—See page 153. Yellow oil drops with **penetrating odor.** Cold, saturated aqueous **solution milky when warmed.** Spontaneous evaporation of trace of coniine with drop of hydrochloric acid leaves **coniine hydrochloride** as doubly refractive crystals that are needle or prism-shaped and sometimes in star-like clusters. **Physiological test:** Paralysis of peripheral nerves; action similar to that of curare.

Nicotine.—See page 156. Liquid. Remains dissolved in residual water upon evaporation of ether and has faint tobacco odor. **Melzer's test:** Red color when heated with 2–3 cc. of epichlorohydrine. **Schindelmeiser's test:** Intense red color after standing several hours with drop of formaldehyde solution upon addition of drop of nitric acid. **Roussin's test:** Ether solution of iodine after long standing with nicotine in closed test-tube gives ruby-red, crystalline needles.

Aniline.—See page 71. Reddish or brownish oil drops upon evaporation of ether extract. Test aqueous solution of these as directed above for aniline (see page 160).

Veratrine.—See page 160. Soluble in concentrated sulphuric acid[2] with yellow color gradually passing into orange, red and finally cherry-red. Gentle heat favors this color change. Solution of vera-

[1] Caffeine behaves like antipyrine. Ether removes only a small part from the tartaric acid solution. The greater part of antipyrine when present will be found in the ether extract B and mainly in the chloroform extract D.

[2] Before a definite conclusion is drawn from the result and course of a reaction with concentrated sulphuric acid, concentrated nitric acid, and the reagents of Erdmann, Froehde, Marquis and Mecke, a careful, comparative study of the behavior of these reagents with the various alkaloids should first be made. (Pages 237 to 238.)

trine in concentrated sulphuric acid shows at first pronounced green-yellow fluorescence. Froehde's reagent produces same color changes. Stable red color when warmed in water-bath with concentrated hydrochloric acid. **Weppen's test:** Mixture of veratrine with about 6 times quantity of cane-sugar gradually colored green and finally blue with few drops of concentrated sulphuric acid. Concentrated sulphuric acid containing furfurol may be substituted for cane-sugar.

Strychnine.—See page 164. Frequently very fine, **intensely bitter,** crystalline needles upon evaporation of ether solution. Small particle of **potassium dichromate** colors sulphuric acid solution of strychnine fugitive blue or blue-violet. Mandelin's reagent produces same color but more stable than that given by potassium dichromate.

Brucine.—See page 171. **Concentrated nitric acid dissolves brucine with blood-red color** changing immediately to red-yellow and yellow. Stannous chloride solution added drop by drop to yellow solution in test-tube converts yellow to violet. Or solution of brucine in very dilute nitric acid may be carefully added as upper layer to concentrated sulphuric acid. Result is red or red-yellow zone.

Atropine.—See page 173. Evaporated in porcelain dish upon water-bath with few drops of fuming nitric acid, atropine remains as yellowish residue that becomes violet when moistened with alcoholic potassium hydroxide solution. Hyoscyamine and scopolamine also give this test. Behavior of strychnine and veratrine similar to that of atropine. **Physiological test** with cat's eye: Even drop of atropine solution diluted 1:130,000 produces dilatation of pupil. **Gulielmo's odor test. Micro-reactions** of Eder.

Cocaine.—See page 185. Precipitated from solutions of salts by potassium hydroxide solution as oil drops that soon solidify and become crystalline. **Permanganate test. Iodic acid test. Detection of benzoyl-group:** If cocaine is warmed in test-tube with 1 cc. of concentrated sulphuric acid for 5 minutes in boiling water-bath and 2 cc. of water are then carefully added, odor of methyl benzoate will be recognized and as solution cools crystals of benzoic acid will appear. Latter may be identified by melting-point (120°) and tendency to sublime. **Physiological test:** Anaesthesia of the tongue.

Codeine.—See page 197. Concentrated sulphuric acid dissolves codeine in cold without color; after standing for some time or at once

when gently warmed, solution takes on reddish or more of bluish color. Solution of codeine in concentrated sulphuric acid gently warmed with potassium arsenate, or instead of the latter with trace of ferric chloride solution, becomes pure blue or blue-violet. Soluble in Froehde's reagent with yellowish color, changing at once to green and upon gentle warming to blue. Codeine dissolves in formalin-sulphuric acid with reddish violet color, immediately changing to stable blue-violet. Codeine gives Pellagri's test (see Morphine).

Narcotine.—See page 201. Reaction not alkaline (distinction from other alkaloids) and taste not bitter. Soluble in Froehde's reagent with green color. If concentrated Froehde's reagent is used (0.05 gram of ammonium molybdate + 1 cc. of concentrated sulphuric acid), color greenish at first gradually changes to fine cherry-red and from the margin to blue. Soluble in Erdmann's reagent with fine red color.

Hydrastine.—See page 205. Soluble in Froehde's reagent with quite stable green color that changes later to brown. Dissolves in Mandelin's reagent with red color, gradually changing to orange-red. Solution of hydrastine in dilute sulphuric acid, shaken with very dilute potassium permanganate solution, exhibits intense blue fluorescence. Permanganate solution should be added drop by drop.

Quinine.—See page 209. Residue from ether solution usually amorphous varnish having very bitter taste. Solution of quinine in dilute sulphuric acid exhibits **blue fluorescence. Thalleioquin test:** About 1 cc. of strong chlorine water first added to solution of residue in dilute acetic acid and then at once drop by drop ammonia in excess; emerald-green color appears, if quinine present. **Herapathite test:** Solution of residue from ether in about 10 drops of mixture of 30 drops of acetic acid, 20 drops of absolute alcohol, and 1 drop of dilute sulphuric acid, heated to boiling and treated with 1 drop of alcoholic iodine solution (1:10). If quinine present, lustrous olive-green leaflets, having cantharides-green appearance in reflected light, separate. **Erythro-quinine test:** One drop each of half-saturated bromine water, potassium ferrocyanide solution (1:10) and ammonia added to 10 cc. of faintly acid, preferably acetic, solution of residue produce with agitation gradually red color. If this mixture is extracted at once with chloroform, this solvent will take on fine violet-red color if quinine present.

Pyramidone.—See page 216. Frequently obtained as fine needles easily soluble in water by evaporating ether solution. Acidified

aqueous solution gives fugitive blue-violet color with little potassium nitrite; this color quickly disappears upon further addition of nitrite. Blue-violet color with ferric chloride solution, or very dilute iodine solution, soon becoming red-violet and then fading. Blue color with silver nitrate solution; later darkening with separation of metallic silver.

Antipyrine and Caffeine.—Detected by means of tests given under A.

Physostigmine.—See page 191. Evaporated with ammonia gives blue residue soluble in alcohol with blue color. **Physiological test:** Contraction of the pupil.

C.[1] Residue from Ether Extract of Aqueous Solution[2] Rendered Alkaline with Ammonia May Contain:

Apomorphine.—See page 219. Residue amorphous and usually greenish. Drop of concentrated nitric acid colors solution in little concentrated sulphuric acid fugitive violet, then red-yellow or orange. Soluble in Froehde's reagent with green or violet color. If solution of apomorphine in dilute hydrochloric acid first treated with excess of sodium bicarbonate and then well-shaken with 1–2 drops of alcoholic iodine solution, it acquires blue-green or emerald-green color, and ether shaken with it takes on violet color. **Wangerin's test:** Solution of apomorphine hydrochloride shaken with 1–2 drops of potassium dichromate solution (0.3 per cent $K_2Cr_2O_7$) gradually turns dark green, and if chloroform added it becomes violet. Upon careful addition of dilute stannous chloride solution, chloroform takes on pure indigo-blue color. **Feinberg's test.**

D. Residue from Chloroform Extract of Aqueous Solution Rendered Alkaline with Ammonia, or Sodium Bicarbonate, May Contain:

Morphine.—See page 225. **Very bitter,** usually amorphous, rarely crystalline, from chloroform solution. **Preliminary test:** Test-portion of residue dissolved in little dilute sulphuric acid, treated with few drops of iodic acid solution, and shaken with chloroform; latter will have violet color. Soluble in Froehde's reagent with

[1] Extraction with ether according to C is necessary only when aqueous solution rendered alkaline with sodium hydroxide solution is deep green and supernatant ether solution is rose or red-violet, that is, when apomorphine is present.

[2] Aqueous solution from B alkaline from sodium hydroxide, after separation of ether extract, is first acidified with dilute hydrochloric acid (test with litmus paper), then rendered alkaline with ammonia, or sodium bicarbonate, and at once thoroughly extracted repeatedly with ether, if necessary, or direct with hot chloroform, or with a hot alcohol-chloroform mixture (1 + 9).

violet color, gradually changing to dirty green and finally to faint red. Soluble in Marquis' reagent with purple-red color, later becoming blue-violet and almost pure blue. **Husemann's test:** Solution of morphine in concentrated sulphuric acid, heated over very small flame until copious fumes of sulphuric acid appear, gives when cold with drop of concentrated nitric acid fugitive red-violet color changing at once to blood-red or red-yellow. **Pellagri's test.** Ferric chloride test: Not too little of the residue from chloroform dissolved in few drops of very dilute hydrochloric acid, solution evaporated upon water-bath to dryness, residue dissolved in little water, and drop of ferric chloride solution added. Appearance of blue color indicates presence of morphine. **Biological test.**

Narceine.—See page 234. Blue color with iodine water. Intensely yellow solution in resorcinol-sulphuric acid exhibits carmine-red, often more of cherry-red, color when warmed upon water-bath and stirred. Yellow-brown solution of narceine in tannin-sulphuric acid becomes pure green when warmed upon water-bath.

Colchicin.—Yellow or more of brownish yellow varnish giving very well tests described for colchicin (see A).

Antipyrine and Caffeine.—These two substances relatively difficulty soluble in ether, but easily soluble in chloroform, also frequently occur, if present, in residue from chloroform extract D and may be recognized by tests described under A.

SYNOPSIS OF GROUP III

Residue left after volatile poisons (Group I) have been distilled off, or portion of original material, is stirred in a glass flask or porcelain dish with dilute hydrochloric acid (10 per cent. HCl). Solid potassium chlorate, or its concentrated aqueous solution, is added and mixture is heated upon boiling water-bath and frequently stirred until most of material has dissolved and liquid has yellow color. Few drops of sulphuric acid are added to solution after dilution with water and it is filtered when cold. In case filtrate contains much free acid, most of excess is removed by evaporation.

Filtrate:
Hydrogen sulphide gas passed through solution, while warmed (1–2 hours) upon water-bath. After standing in loosely stoppered flask, best until following day, solution filtered.

Residue:
Metallic Poisons IV (page 262)
Ag, Pb, Ba.

↓

Precipitate:
Hot ammonia-ammonium sulphide mixture poured repeatedly over filter.

Filtrate:
Metallic Poisons III (page 260).
Cr, Zn.

↓

Filtrate:
Metallic Poisons I (page 249).
As, Sb, Sn, Cu.

Residue:
Metallic Poisons II (page 257).
Hg, Pb, Cu, Bi, Cd.

CHAPTER IV

POISONS NOT IN THE THREE MAIN GROUPS

A. INORGANIC SUBSTANCES

Mineral Acids

As a rule, examination of cadaveric material for mineral acids is necessary only when the autopsy points conclusively to such poisoning. That is, when characteristic corrosions and discolorations about the face, mouth, oesophagus and stomach are visible. Since chlorides, nitrates and sulphates are normal constituents of nearly all animal organs and fluids, proof must be given that free mineral acid is present.

To detect free mineral acid, extract a portion of material with cold water, filter and test as follows, if the solution is strongly acid:

1. **Methyl Violet Test.**—Add a few drops of an aqueous (0.1:1000), or alcoholic (1:100), solution of methyl violet to a portion of the filtrate. Free mineral acid produces a blue or green color. Organic acids are without action.

2. **Methyl Orange Test.**—Add a few drops of dilute aqueous solution of methyl orange to a portion of the filtrate. A red color indicates free mineral acid.

3. **Congo Paper Test.**—Even very dilute solutions of free mineral acids turn "Congo paper" blue. Organic acids are without action.

4. **Günzburg's Test.**—Mix a few drops of the filtrate with 3–4 drops of Günzburg's reagent[1] and evaporate to complete dryness upon the water-bath, or over a small flame. Free hydrochloric or sulphuric acid gives a fine red or reddish yellow residue. Nitric acid gives more of a yellowish red residue.

If general tests show presence of free mineral acid, make special tests for the particular acid.

Hydrochloric Acid

1. **Chlorine Test.**—Warm a little of the aqueous extract, not too dilute, with finely powdered manganese dioxide. Free hydrochloric

[1] See "Preparation of Reagents," page 642.

acid yields chlorine, recognized by color and odor, or by passing the gas into potassium iodide solution and liberating iodine. Hydrochloric acid exclusively does not give this test. A chloride and free sulphuric acid give chlorine under the same conditions. Free bromine will also liberate iodine from potassium iodide.

2. **Distillation.**—If possible, separate hydrochloric acid from other substances by distillation and test the distillate. Concentration of the acid is especially important, since dilute hydrochloric acid upon distillation at first yields only water. Hydrochloric acid[1] itself does not begin to distil until concentration is about 10 per cent. Since dilute hydrochloric acid is usually examined, distil the material mixed with water, or preferably a filtered aqueous extract, nearly to dryness. In such distillation apply heat by means of an oil-bath. To detect hydrochloric acid in the distillate, acidify with dilute nitric acid and add silver nitrate solution. Frequently a quantitative estimation of hydrochloric acid is required. In absence of other acids, titrate the distillate with 0.1 n-sodium hydroxide solution, using phenolphthalein as indicator. Otherwise, estimate the acid gravimetrically, precipitating with silver nitrate and weighing silver chloride, or volumetrically by Volhard's method. Since the human stomach normally contains 0.1–0.6 per cent. of free hydrochloric acid, examination of stomach-contents for this acid must always include a quantitative estimation. A conclusion that poisoning by hydrochloric acid has taken place is justifiable only when a large quantity of acid is found, provided also that the autopsy unmistakably indicates its presence.

Nitric Acid

The human body normally contains only a very small amount of nitrates. When present, they owe their origin usually to drinking water and vegetable foods. Human urine almost always shows traces of salts of nitric and nitrous acids. Chemical examination of cadaveric material need not include tests for nitric acid, unless the autopsy affords evidence of poisoning by this acid, such as distinct signs of corrosion about lips, mouth, oesophagus and stomach and sometimes perforation. These parts are more or less yellow or yellowish brown. Yellow froth is said to exude from the mouth

[1] If, for example, 100 cc. of 1 per cent. hydrochloric acid are distilled, the first 90 cc. of distillate will contain only traces of acid. Almost all acid will be found in the last portion of distillate.

and nose of the cadaver. Also stomach-contents are yellow in concentrated nitric acid poisoning. If concentration of acid is less than 20 per cent., these specific changes may not appear in the gastro-intestinal tract. Nitric acid taken internally, dilute or concentrated, appears at once in the urine.

Detection of Nitric Acid

1. **Distillation.**—If possible, extract material direct with water, filter and test the filtrate for nitric acid in the usual way. When the quantity of nitric acid is large, separate it from other substances by distilling the filtered aqueous extract. Apply heat by means of an oil-bath. Like hydrochloric acid, nitric acid[1] does not distil until a definite concentration is reached. The filtered extract should be distilled nearly to dryness. Considerable nitric acid is lost, for it combines with organic substances, particularly proteins, forming nitroderivatives, xanthoproteic acid, etc. Nitric acid may also cause oxidation. So the distillate will not contain all the acid originally present. Toward the end of distillation brown vapors of nitrogen peroxide often appear. Such a distillate, added to starch paste and potassium iodide, produces an immediate blue color in presence of dilute sulphuric acid. The residue from distillation is usually distinctly yellow.

The following tests serve to detect nitric acid in the distillate:

2. **Fleury's Procedure.**[2]—Nitric acid, according to Fleury, varies in effect upon the human body. Sometimes it stains epithelial tissue yellow, having merely a superficial action; again in contact with muscle tissue it forms an eschar, or converts it into a cheesy mass through coagulation of albumin.

Positive detection of nitric acid poisoning is more difficult than generally supposed. To detect free nitric acid still present unchanged, extract the finely divided material with three times the quantity of alcohol, filter and add slaked lime in excess to the filtrate. To decompose any nitric acid ester present, allow the mixture to stand for 12 hours, filter and evaporate the filtrate to dryness. Dissolve the residue in 95 per cent. alcohol, expel alcohol from the filtered

[1] If a thin magma of crumbled dog-biscuit and 100 cc. of 1 per cent. nitric acid are distilled, by far the greater part of acid will be found in the last 10 cc. of distillate.

[2] G. Fleury: Toxicological Detection of Nitric Acid. Ann. Chim. analyt. appl. 6 (1901), 12; Rep. de Pharm. 56 (1907), 385.

solution, and finally test an aqueous solution of the residue for nitric acid. By this method Fleury obtained about 20 per cent. of the nitric acid from animal material. This procedure converts the acid into the calcium salt which is soluble in 95 per cent. alcohol. But sodium nitrate is also quite soluble (1:50) in alcohol of the same strength. So if the final residue gives a faint test for nitric acid, proof of free acid in the original material is not conclusive. The method of Baumert obviates this difficulty.

3. **Baumert's Procedure.**[1]—Neutralize the material itself, or its aqueous extract, with milk of lime, dry and extract with alcohol; or, after neutralization with milk of lime or calcium carbonate, evaporate to a syrup, stir and mix with alcohol. Distil the filtered alcoholic extract obtained in either way, dissolve the residue in water, filter and evaporate the solution. Dissolve the residue again in alcohol and allow this solution to stand for several hours in a closed flask with about the same volume of ether. Filter this alcohol-ether solution, evaporate the solvent, and dissolve the residue in a little water. Apply the following nitric acid tests to this solution:

(a) **Diphenylamine-Sulphuric Acid Test.**—Add a few drops of diphenylamine sulphate solution[2] to the aqueous extract, or distillate, and carefully pour this mixture upon pure concentrated sulphuric acid free from nitric acid. If nitric acid is present, a blue zone appears where the two liquids meet.

(b) **Brucine-Sulphuric Acid Test.**—Mix the same volume of brucine sulphate solution[3] with the liquid to be tested and carefully pour this mixture upon pure concentrated sulphuric acid. If nitric acid is present, a red zone appears where the two liquids meet.

(c) **Ferrous Sulphate-Sulphuric Acid Test.**—Saturate the liquid to be tested with pure ferrous sulphate and carefully pour this solution upon concentrated sulphuric acid. If nitric acid is present, a black zone appears where the two liquids meet.

[1] G. Baumert: Lehrbuch der gerichtlichen Chemie. Second edition, 1907.

[2] Solution prepared from 1 gram of diphenyl-amine, 5 grams of dilute sulphuric acid, and 100 cc. of water.

[3] Solution prepared from 1 gram of brucine, 5 grams of dilute sulphuric acid, and 100 grams of water. The sulphuric acid used in preparing both these reagents should not itself give the test. Should it do so, the sulphuric acid must be heated in a platinum dish until nitrous acid is completely expelled; or the acid may be distilled from a small retort, when nitric and nitrous acid will be found in the first part of the distillate which should be rejected.

(d) **Copper Test.**—Place a small piece of clean copper (wire or sheet) in nitric acid and heat. Red-brown vapors of nitrogen peroxide (NO_2) appear.

Sulphuric Acid

Nearly all animal and vegetable substances normally contain sulphates. So an examination for free sulphuric acid must exclude its salts. It is useless to examine cadaveric material for free acid, unless marked corrosion and discoloration of lips, mouth, oesophagus, and stomach indicate its presence. There are eschars upon the lips and the mucous lining of the mouth is grayish white. The white coating on the back of the tongue may have been dissolved, exposing the firm, brownish muscular tissue beneath. The tongue often looks as if it had been boiled. The mucous lining of the oesophagus is much wrinkled and coated gray. Externally the stomach is usually brown, or slate-gray, and its contents black. Frequently in sulphuric acid poisoning there is perforation of the stomach wall and brownish black masses find their way into the abdominal cavity. There may be black spots in the stomach, due according to R. Kobert (Intoxikationen) not to charring, as previously supposed, but to brown-black haematin. Acids decompose the blood-pigment oxyhaemoglobin mainly into haematin and protein (globulin). Methaemoglobin and haematoporphyrin may also be formed. Acids produce the latter from haematin and in the change there is loss of iron. All three of these decomposition products of the red blood-pigment, namely, methaemoglobin, haematin and haematoporphyrin may be formed successively and then appear in the urine. The blood in the stomach walls is often acid and then contains chiefly methaemoglobin and haematin. The mucosa of the intestines even far down may be grayish white and strongly acid.

Detection of Sulphuric Acid

1. Extract the finely divided material, if strongly acid, with cold absolute alcohol and after some time filter. The solution contains sulphuric acid but not sulphates. Evaporate the alcoholic filtrate upon the water-bath, or, if the volume is large, distil the alcohol. Dissolve the residue in 10 cc. of water and heat the solution to boiling to saponify ethyl sulphuric acid. Filter and test the filtrate with barium chloride or lead acetate solution. To prove that the precipitate is a sulphate, mix with sodium carbonate and fuse upon charcoal. Sodium sulphide formed blackens metallic silver in presence of water, or gives hydrogen sulphide with acids.

2. Extract the finely divided material with water and apply the following tests to the filtrate:

(a) **Sugar Test.**—Evaporate some of the filtered extract in a porcelain dish with a small particle of sugar. Free sulphuric acid produces a black, carbonaceous residue.

(b) **Sulphur Dioxide Test.**—Concentrate the filtered extract upon the water-bath and heat in a test-tube with a few pieces of copper. Free sulphuric acid generates sulphur dioxide, recognized by its stifling odor. Distil sulphur dioxide (preferably in an atmosphere of carbon dioxide) into a little water and test the distillate as follows:

α. Warm a portion of liquid with a little stannous chloride solution. A yellow precipitate of stannic sulphide[1] appears.

β. Add iodo-potassium iodide solution drop by drop. The color of iodine disappears and at the same time sulphuric acid is formed. Barium chloride then precipitates barium sulphate insoluble in dilute hydochloric acid.

To estimate sulphuric acid quantitatively, either precipitate and weigh barium sulphate in the usual way, or titrate with 0.1 n-sodium hydroxide solution, using phenolphthalein as indicator.

1000 cc. of 0.1 n-sodium hydroxide solution = 0.1 gram-equivalent of sulphuric acid = 4.9 grams of H_2SO_4. In the latter method no other acid except sulphuric should be present.

Hydrofluoric Acid and Soluble Fluorides

Hydrofluoric acid is a powerful, corrosive poison. In producing toxic phenomena due to corrosion and acidification, its vapor is quite as effective as that of hydrochloric acid. Of water soluble, metallic fluorides, sodium fluoride in particular has been tested thoroughly as to toxic action. This salt finds use in medicine. Tappeiner has found sodium fluoride a protoplasm poison. Very likely it acts by withdrawing calcium. Applied to the cornea, it produces ulcers, subcutaneous necrosis and suppuration. Injected into the blood, it paralyzes the central nervous system, causing at the same time muscular twitching and convulsions. Coagulation of blood is also prevented, for calcium needed in this reaction is precipitated through combination with fluorine. Taken by the mouth, sodium fluoride may cause inflammation of the mucous lining of the stomach. Action of the heart is weakened and blood-pressure falls. Death finally takes place from paralysis of the respiratory centre. Sodium fluoride exerts a strong antiseptic action upon bacteria. Baldwin[2] cites the case of several persons made very ill from eating cake in the preparation of which sodium fluoride was used by mistake for baking powder. The person who had eaten most of the cake died on the same day.

To detect water-soluble fluorides in such material as gastric or intestinal contents, extract with water, filter and precipitate with calcium chloride. Collect the precipitate upon a filter, dry (best by gentle ignition in a crucible), and make the following tests for fluorine:

(a) **Etching Test.**—Pour a little concentrated sulphuric acid upon some of the dried precipitate in a platinum crucible. Cover with a watch-glass coated upon one side with paraffine and then written upon. Allow the watch-glass to remain over the crucible for about one hour. Then remove the paraffine coating. Etching of glass exposed by the writing shows presence of fluorides.

[1] Sulphurous acid and sodium sulphite, added to stannous chloride solution not too strongly acid, precipitate stannous sulphite, $SnSO_3$, white and readily soluble in hydrochloric acid. Warmed in presence of hydrochloric acid, sulphur dioxide acts upon a stannous salt as an oxidizing agent. A precipitate of $Sn_6O_{10}S_2$ is formed, or H_2S is evolved and $SnCl_4$ formed, depending upon the amount of hydrochloric acid present. (Prescott and Johnson, Qualitative Chemical Analysis. Fifth edition, page 86.)

[2] H. B. Baldwin: Journ. of the Amer. Chem. Soc. 21, 517.

(b) **Silicic Acid Test.**—Mix the well-dried precipitate with a little sand and heat with concentrated sulphuric acid in a dry test-tube. Bring a glass rod having on its end a drop of water in contact with the vapor given off; or pass the vapor into a larger moist test-tube. In presence of hydrofluoric acid the drop of water solidifies because of separation of silicic acid more or less gelatinous, and a white efflorescence also consisting of silicic acid forms upon the moist part of the test-tube.

Explanation of Reaction.—Hydrofluoric acid set free from the fluoride by sulphuric acid dissolves the sand forming gaseous silicon tetrafluoride. Water decomposes the latter into silicic acid, which separates gelatinous, and soluble fluosilicic acid;

$$\alpha.\ 2NaF + H_2SO_4 = Na_2SO_4 + 2HF,$$
$$\beta.\ SiO_2 + 4HF = SiF_4 + 2H_2O,$$
$$\gamma.\ 3SiF_4 + 4H_2O = \underset{\text{Gelatinous precipitate}}{H_4SiO_4} + \underset{\text{In solution}}{2H_2SiF_6}.$$

Official Directions (German) for Detection of Hydrofluoric Acid and Salts in Meat

Thoroughly mix 25 grams of finely divided meat in a platinum dish with sufficient milk of lime. Then dry, ash and transfer the powdered residue to a platinum crucible. Moisten the powder with 3 drops of water and add 1 cc. of concentrated sulphuric acid. After adding the sulphuric acid, at once cover the crucible with a large watch-glass and heat upon an asbestos plate. The convex side of the watch-glass should be covered with paraffine and the glass exposed by writing. Put a piece of ice in the watch-glass to keep the paraffine from melting. If the glass exposed by the writing is etched, presence of hydrofluoric acid is proved and the meat is regarded as having been treated with hydrofluoric acid or fluorides.

Bromine and Alkali Bromides

Free bromine or its vapor colors the skin brown and may cause blisters and necrosis. It corrodes mucous membranes causing intense burning pain. No matter where applied, bromine after absorption gives rise to nervous depression, stupor and narcosis. Bromine vapor inhaled causes inflammation of the conjunctiva, lachrymation, coryza, salivation, distressing attacks of coughing, pseudo-asthma and bronchitis. At the same time there may be abdominal pains, diarrhoea and vomiting. The most frequent manifestations are giddiness, headache and stupor (R. Kobert). Severe disturbances of a general character may result from long-continued use of large quantities of alkali bromides (sodium, potassium or ammonium bromide). Chronic bromism manifests itself in coryza, coughing, heaviness in the head, weakening of the mental faculties, disturbance of speech, sexual impairment, but particularly in skin eruptions (bromine acne) which may become malignant. The secondary effects due to alkali bromides resemble in a way those caused by potassium iodide but are ordinarily more persistent than the latter. Bromides attack mucous membranes more easily than iodides do. Moreover the intensity and duration of the effect of bromides upon the skin, and in still higher degree upon the central nervous system, are greater than in the case of iodides. As a rule, the range of tolerance of alkali bromides is not very wide.

Resistance of children 8–14 years of age, as a rule, is said to be higher than that of adults. Individuals have used alkali bromides for years without appearance of secondary effects. But in other cases these have appeared after very small quantities have been taken. Lewin[1] cites the case of a man who took 2 grams of potassium bromide and lapsed into a state in which he believed he dreamed everything he saw, heard and felt. The way in which absorption takes place is immaterial. Whether introduced by way of the stomach, by inhalation, or by the rectum bromides may give rise to secondary effects.

Alkali Bromides in the Human Body

Absorption of alkali bromides from the mucous membranes is rapid. But elimination is much slower. The human body stubbornly retains sodium bromide taken by the mouth and eliminates it through the kidneys very slowly.

In an experiment by Autenrieth,[2] an adult with healthy kidneys, non-epileptic, on a mixed diet, and with continuously good diuresis, took two doses of 4 grams each of sodium bromide and eliminated in the course of 35 days 5.68 grams in the urine, or 71.8 per cent. On the 33d day after the last dose 10 mg. of sodium bromide were found in the 24 hour urine. In this as well as in other experiments it was found that very little sodium bromide, only 3.2 per cent. of that taken, was eliminated on the first two days after medication. Elimination attained its maximum on the third day. The ability of two persons with diseased kidneys to eliminate sodium bromide, as compared with the person having healthy kidneys, was far less. The fact, however, must be taken into consideration that the two persons with diseased kidneys were on a diet low in salt. An instance given by Autenrieth[3] is in full agreement with these experimental results. Parts of the cadaver of a man 37 years old, ill from chronic nephritis, were examined. He had been on a diet low in salt and had taken daily for 10 days at least 15 grams of sodium bromide. Then during 28 days prior to death he had taken no bromide. After this long period without bromide the blood and different organs of this man had retained a considerable quantity, that is, liver 0.037, brain 0.019, kidneys 0.037, and blood 0.045 per cent. of bromine calculated as sodium bromide, and for the organ containing all its blood. Even after large quantities of sodium bromide have been taken the brain does not show an increase in bromine.

Detection and Estimation of Bromine

To detect free bromine, for example, in stomach-contents, or in organs of the body, the material should be first distilled with water. A case, however, where this is necessary would rarely occur, for free bromine would at once combine chemically with the organic substances forming bromine substitution products and hydrobromic acid. Should simple distillation fail to show bromine, add

[1] L. Lewin: "Secondary Effects of Drugs." Third edition, 1899, page 157.

[2] W. Autenrieth: Elimination of Bromine from Man following Administration of Sodium Bromide. Münch. med. Wochenschr. 1918, page 749.

[3] W. Autenrieth: Estimation and Distribution of Bromine in Organs and Blood Following Administration of Sodium Bromide. Münch. med. Wochenschr. 1918, page 33.

excess of potassium bichromate to the material, render strongly acid with dilute sulphuric acid, and distil again. But even this procedure is not always successful. In the experiments mentioned above, Autenrieth found a fusion method advantageous. He tested for bromine qualitatively and at the same time determined it quantitatively by the colorimetric method.

Ashing of Urine and Colorimetric Estimation of Bromine in Ash

Estimation of bromine colorimetrically in alkali and other bromides depends upon the reaction taking place in presence of potassium permanganate and dilute sulphuric acid, or better acid potassium sulphate solution. Bromine is completely set free and taken up without loss in a measured volume of chloroform. By means of a calibrated comparison-wedge the tinctorial strength of this solution is determined in the Autenrieth-Königsberger colorimeter.[1]

α. $2NaBr + H_2SO_4 = Na_2SO_4 + 2HBr$

β. $10HBr + 2KMnO_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 5Br_2 + 8H_2O$

Procedure.—According to the probable quantity of bromine present, take 20, 50, 100 or more cc. of urine. Add 1 gram of sodium carbonate and 0.5–1 gram of potassium nitrate and evaporate the measured volume to dryness in a rather deep nickel crucible of sufficient capacity to be only half-full. Near the end of evaporation heat the crucible cautiously with a smaller flame, to avoid loss from spirting due to too vigorous deflagration. Application of heat on the side rather than bottom of the crucible is advisable. In this manner urine may be quickly evaporated without frothing over. Further heating after sprinkling a little finely powdered potassium nitrate over the dry residue, best while rotating the crucible, will remove any particles of carbon and bring about complete fusion of the mass. Of course the melt should not be heated too intensely nor needlessly long. But even then there is hardly any loss of sodium bromide, for its vapor tension is low and any bromide in the vapor phase is at once precipitated on the cooler parts of the deep covered crucible. Usually 2–3 grams of potassium nitrate are enough for complete combustion of carbon particles in the ash from 100 cc. of urine. Soften the cold melt with 10–15 cc. of water (sodium bromide is so soluble that more water is not needed) and neutralize the solution in the crucible with dilute sulphuric acid so that red litmus paper is turned distinctly blue. Filtration of the usually turbid solution is not necessary. Transfer to a separating funnel and without loss rinse the crucible with 10–20 cc. of 10 per cent. acid potassium sulphate solution. According to the probable quantity of bromine in the urine, add 20–30 cc. of pure chloroform and 3 per cent. potassium permanganate solution drop by drop until the color is no longer discharged. Finally add 15–20 drops more of the latter solution and allow the separating funnel, stoppered and protected from light, to stand for 15 minutes at rest. Then shake well and, as soon as the chloroform solution of bromine has settled, run as completely as possible into a dry glass-stoppered graduate.

[1] A description of this colorimeter and more detailed information as to its use will be found in Chapter V of this book (see page 468). The comparison-wedges required for bromine determinations may be procured from the Firm of C. Hellige and Co., Freiburg i.B.

Using the same volume of chloroform, shake again, and if much bromine is present even a third portion, and run the extracts into the graduate. Finally dilute the combined extracts with chloroform to a definite volume. Should the chloroform solution of bromine not be entirely clear, shake with pieces of filter paper, or pour quickly through dry paper. With this clear solution, poured into the small glass trough of the colorimeter, determine the color strength as described below for calibration of the comparison-wedge. The quantity of potassium bromide, corresponding to the color strength of the chloroform solution of bromine examined, is determined from the calibration curve of the comparison-wedge by looking on the abscissa for the point in the curve corresponding to the same strength of color indicated by the reading on the scale of the colorimeter. This gives the mg. of potassium bromide corresponding to bromine in each 10 cc. of chloroform solution of bromine. To calculate the quantity of sodium bromide, multiply the quantities of potassium bromide derived from the curve by 0.8647: NaBr: KBr = 102.92: 119.02 = 0.8647. Organs of the body, such as liver, or brain, as well as blood, should be finely divided, well-mixed in a nickel crucible with sodium hydroxide prepared from metallic sodium and triturated. Carbonize this material and finally ash it completely by means of potassium nitrate as described above.

Example of Calculation.—24 hour urine: 3300 cc. Volume for bromine determination: 100 cc. Volume of chloroform solution of bromine: 40 cc. Identical color strength: scale reading 58 = 7.9 mg. of KBr, corresponding to bromine in 10 cc. of chloroform solution of bromine. So 40 cc. of the latter solution, or 100 cc. of urine, contain $4 \times 7.9 = 31.6$ mg. and the 24 hour urine $33 \times 31.6 =$ 1042.8 mg. = 1.0428 grams of KBr. This quantity of KBr corresponds to $1.0428 \times 0.8647 = 0.902$ gram of NaBr.

Notes.—Larger volumes of urine, 200 cc. and more, should first be evaporated to smaller volume in a porcelain dish over free fire. Evaporation to dryness and ashing should be carried out in a nickel crucible. The mixture of bromide, acid potassium sulphate and potassium permanganate should stand at least 15 minutes, otherwise the results are liable to be too low. Addition of chloroform to this mixture before extracting bromine gradually set free is advisable, otherwise opening the separating funnel and pouring in chloroform may result in loss of bromine from evaporation.

Calibration of Comparison-wedge.—To determine bromine colorimetrically by the Autenrieth-Königsberger colorimeter, fill a glass-stoppered wedge with a chloroform solution of bromine of such dilution that the lower part of the wedge still has a perceptible yellow color. This is the comparison-wedge and is calibrated with potassium bromide solution of known strength. This is done by introducing different but exactly measured volumes of 1: 1000 potassium bromide solution into a rather small separating funnel. Each time add 5 cc. of dilute sulphuric acid (1: 10) and enough water to have the same volume in all cases, for example, 30 cc. Finally add to each portion 10 cc. of pure chloroform and 12–15 drops of saturated potassium permanganate solution. Stopper the separating funnel and allow the mixture to stand protected from light for 10 minutes. Then shake well and pour a portion of the clear chloroform solution of bromine into the small glass trough of the colorimeter. By adjusting the comparison-wedge, determine on the scale where the color strength of the two

solutions is the same. If the chloroform solution of bromine is not clear, shake with filter paper or pour through a small dry filter.

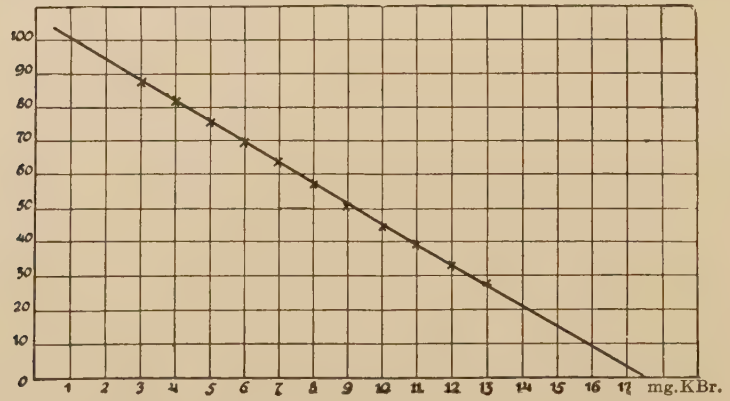


FIG. 23.—Calibration curve of comparison-wedge for colorimetric determination of bromine in bromides. Ten cc. of Br-$CHCl_3$ solution for each reading.

By the calibration of the comparison-wedge in this manner the following values were obtained:

Solution in Cc.	KBr. in Mg.	Scale-reading
4	4	81–82
5	5	76–77
7	7	63–64
9	9	50–51
10	10	44–45
11	11	40–41
12	12	33–34
13	13	28–29

The calibration-curve (Fig. 23) of the comparison-wedge is obtained by plotting each time the quantity of potassium bromide in mg. upon the abscissae and the reading on the colorimeter scale for equal color strength upon the ordinates.

Iodine and Iodides

Fairly large quantities of free iodine, for example, tincture of iodine, taken internally, give rise to pain in the mouth, oesophagus and stomach. Vomiting of dark yellow masses, or blue in case the food in the stomach contains starch, may occur as well as diarrhoea, bloody stools and collapse. In rare cases poisoning terminates fatally. A laborer who had drunk 58 grams of tincture of iodine = 5.8 grams of iodine collapsed on the second day and died after 33 hours (R. Kobert). Frequently skin eruptions and diarrhoea result from painting the skin with tincture of iodine. Phenomena that seem to indicate severe potassium iodide poisoning are possible, especially if the mucous surfaces of the throat or the female genitals are painted with tincture of iodine or with Lugol's solu-

tion. Kobert places the smallest lethal dose of free iodine at 3 grams. When fatal doses of iodine have been taken, death may ensue in 1–2 days, or even later.

In contact with tissues and fluids, iodine disappears, for it combines both with alkalies and protein substances. Alkalinity of blood-serum is thereby lowered. With iodine living protoplasm forms various protein compounds some of which are stable. Red blood-corpuscles are haemolyzed and excess of iodine carries decomposition of dissolved haemoglobin down to haematin. Free iodine kills living cells of organs, for example, those of the liver or kidneys.

After use of potassium iodide, iodism temporarily appears, manifesting itself by metallic taste, flow of saliva, coryza, dizziness, red and watery eyes. Especially skin eruptions (iodine acne) appear, behaving much like bromine acne. Iodine is present in the contents of the pustules.

Iodine is rapidly eliminated from the blood through the urine after use of potassium iodide. Experiments of the author, where 4 grams of this salt were taken, showed that 78 per cent. was recovered in the urine after 2 days. The thyroid gland retains part of the iodine. Although alkaline iodides are very quickly eliminated from the blood, even after 8 days, Terrile found appreciable quantities of iodine in the testicles which like the thyroid gland appear to be receptacles of this halogen.

Detection of Free Iodine.—Poisoning from free iodine is often recognizable from brown spots upon the skin or upon wearing apparel. These cannot be mistaken for nitric acid spots, for the latter unlike iodine spots are usually pure yellow and do not gradually disappear with ammonia, potassium hydroxide, or sodium thiosulphate solutions but become dark orange or at least remain unchanged. Because of slight affinity for hydrogen, free iodine persists longer than other halogens and may be recognized by color, odor and violet-colored chloroform solution. Gadamer recommends passing a current of air through the gently warmed material and then through chloroform (violet color), or through starch solution (blue color). Moreover in case of severe, particularly fatal poisoning from free iodine, as well as from tincture, it is to be expected that alcoholic extracts of the material will always contain iodine in inorganic combination, that is, ionic iodine.

Detection and Estimation of Iodine in Urine, Blood and Organs after Administration of Alkali Iodides

Usually a qualitative test for ionic iodine in urine may be made direct. Acidify 20–100 cc. of urine with dilute sulphuric acid, add sodium or potassium nitrite solution, and then shake with chloroform. If the latter has a violet color, presence of iodine is shown.

The colorimetric method is more convenient when a quantitative estimation of iodine in organs and fluids of the body must be made. As stated above, alkali iodides taken internally are eliminated rather quickly from the blood through the urine. Usually therefore only small quantities of iodine are found in blood and organs of the body. When it is a question of estimating very small quantities of any substance, colorimetric methods should receive first consideration. Autenrieth and Funk[1] adopted the following procedure with the Autenrieth-

[1] W. Autenrieth and A. Funk: Estimation of Iodine in Urine and Organs. Münchener mediz. Wochenschrift 1912, Nos. 49 and 50.

Königsberger wedge-colorimeter: A comparison-wedge is first prepared by diluting a chloroform solution of iodine with chloroform until the thin end of the wedge when full shows a perceptible color. This comparison-wedge should be kept in the dark.

Calibration of Comparison-wedge.—This wedge, filled and kept well closed, serves as a comparison-wedge in the colorimetric estimation of iodine. First it must be calibrated. In a tared weighing-glass tightly closed with a glass stopper weigh accurately 0.05 gram, or any other aliquot quantity, of purest, dry resublimed iodine. Dissolve the iodine in choroform to a volume of 200 cc. By means of a capillary pipette or burette accurately measure varying volumes of this chloroform solution of iodine, that is, 1, 2, 3 cc., diluting in each case with chloroform to 10 cc. in a small graduate. In each case pour a test portion of the chloroform solution of iodine of known strength into the small glass-stoppered trough of the colorimeter. Adjust the glass wedge and determine the reading where the color of the solution in the wedge has the same intensity as that of the

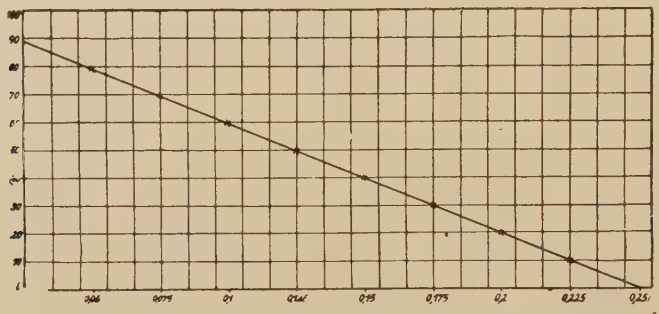


FIG. 24.—Iodine curve. In 1 cc. of chloroform solution.

solution in the trough. For accuracy it is advisable to repeat this 5–6 times and take the average reading. To construct the calibration-curve, take millimeter paper and mark upon the abscissae the quantity of iodine contained in each case in 1 cc. of chloroform solutions examined and upon the ordinates the reading on the colorimeter scale for equal color strength. The line connecting the points thus recorded is the calibration-curve of the comparison-wedge = Iodine Curve Fig. 24.

In the calibration of the comparison-wedge by means of a chloroform solution, containing 0.25 mg. of iodine per cc., the following intersection points leading to the construction of Fig. 24 above were determined for equal color strength:

Chloroform Solution of Iodine		Pure Chloroform	Iodine in 1 Cc. of Solution	Scale Reading for Equal Color Strength
1 cc.	+	9 cc.	0.025 mg.	89
2 cc.	+	8 cc.	0.050 mg.	79
4 cc.	+	6 cc.	0.10 mg.	58
6 cc.	+	4 cc.	0.15 mg.	38
8 cc.	+	2 cc.	0.20 mg.	19
10 cc.	+	0 cc.	0.25 mg.	0

So this comparison-wedge has a range from 0.025 to 0.25 mg. of iodine dissolved in each case in 1 cc. of chloroform.

Estimation of Iodine in Thyroid Gland

E. Baumann's procedure offers a convenient method of carrying out this determination. To get a true average sample, finely comminute the entire thyroid gland, dry at 100° to constant weight, and grind as finely as possible in a small mill. Carefully evaporate 1 gram of this powdered thyroid gland in a clean nickel crucible with about 1 gram of sodium hydroxide prepared from metallic sodium and 5 cc. of water. Completely ash carbon particles and any organic matter still present by sprinkling a little powdered potassium nitrate over the mass and fusing to a white melt. Heating should not be too intense nor too prolonged, otherwise traces of alkali iodide may volatilize. Use a rather deep nickel crucible and keep covered. Dissolve the nearly cold melt in hot water, filter the solution and rinse the crucible into a small separating funnel. According to the quantity of iodine present, add 5, 10, 20 or more cc. of chloroform and acidify with dilute sulphuric acid, shaking carefully, cooling and allowing liberated carbon dioxide to escape. As soon as most of the latter has passed off, shake well to bring about complete solution of iodine in chloroform. Run the clear chloroform solution of iodine through a dry filter into a graduate. Make two more extractions of the solution in the separating funnel with a little chloroform, passing these extracts through the same filter into the graduate. Mix the combined extracts and determine the volume of the chloroform solution of iodine. Pour a test portion of this solution into the glass-stoppered trough of the colorimeter and by means of the calibrated comparison-wedge determine the point at which its color intensity is the same as that of the solution in the trough. Take the reading on the colorimeter scale and ascertain on the iodine curve the value corresponding to the reading. This gives the quantity of iodine in mg. in 1 cc. of chloroform solution. Should the color of the chloroform solution of iodine be too intense, dilute further with chloroform, note the new volume and take another reading.

Estimation of Iodine in Urine

A direct quantitative determination of iodine in urine in the manner described above for the qualitative detection is possible, when urine contains only inorganic combined, that is, ionic iodine, as it does after alkali iodides have been taken internally, provided the acidified urine imparts no color to chloroform. Obviously the volume of the chloroform solution of iodine must be carefully measured. If a direct determination is not possible, evaporate 10 cc. or more of urine to dryness with 1–2 grams of sodium hydroxide prepared from metallic sodium in a clean nickel crucible of suitable size. Completely remove carbon particles and any organic matter by sprinkling 1–2 grams of powdered potassium nitrate over the residue and fusing to a white melt. Heating should not be too intense nor too prolonged. In other respects treatment of the filtered aqueous solution of the melt is identical with that described for determination of iodine in thyroid gland.

If pure sodium hydroxide is not available, evaporate the measured volume of urine with 1 gram of pure sodium carbonate and 2–3 grams of potassium nitrate. In this case fusion of the residue should be conducted at gentle heat over an ordinary Bunsen flame until no carbon particles remain and the melt is white.

To express iodine in terms of potassium or sodium iodide, multiply the value obtained for free iodine by 1.309 for potassium iodide: KI:I = 166.02:126.92 = 1.309; or by 1.19 for sodium iodide: NaI:I = 149.92:126.92 = 1.19.

Note.—Since commercial sodium hydroxide, fused caustic soda, often contains traces of sodium iodide, sodium hydroxide prepared from metallic sodium should be used in the above experiments.

Hydrogen Sulphide

Hydrogen sulphide, H_2S, is one of the most toxic substances known. A person inhaling air containing several per cent. of hydrogen sulphide drops within a few seconds, becomes unconscious, and dies almost immediately without first having convulsions. This is the so-called apoplectic form of hydrogen sulphide poisoning. Most persons can tolerate only 0.01 per cent. of hydrogen sulphide in air. In three cases Lehmann[1] observed that very painful irritation of eyes, nose and throat appeared within 5–8 minutes in an atmosphere containing 0.02 per cent. of hydrogen sulphide, and after 30 minutes their condition was hardly endurable. Air containing 0.05 per cent. of hydrogen sulphide is as much as a man can stand, for after 30 minutes in addition to pain in the eyes it gives rise to nasal catarrh, difficulty in breathing and coughing, also palpitation of the heart, dizziness, unsteady gait, trembling of the extremities, extreme languor, pallor, cold sweat, headache and unconsciousness. Lehmann states that a man in air containing 0.07–0.08 per cent. of hydrogen sulphide will become dangerously ill after several hours and in 0.1–0.15 per cent. will die rather quickly. In some cases symptoms of hydrogen sulphide poisoning do not develop until later, after persons apparently in good health have left the polluted atmosphere. Signs of chronic poisoning in the case of workmen exposed almost daily to hydrogen sulphide appear in the form of conjunctivitis (inflammation of the conjunctiva), headache and gastric disturbances. The complexion is pallid and the skin shows a tendency to furunculosis. Habituation to the poison appears not to take place. On the contrary, apparently the sensitiveness of a person to hydrogen sulphide increases. The findings of the autopsy vary. In case of fatal poisoning terminating rapidly they may be entirely negative. When death comes on slowly, a characteristic odor is noticeable in the body-cavities and sometimes in the organs. In case of an immediate autopsy a greenish change of color of the entire cadaver, or at least of the brain or blood, is not noticed but appears some time after burial and then is very characteristic. Every normal cadaver, however, shows this coloration as a result of normal putrefaction but it is longer in appearing!

Detection in Parts of the Cadaver

Only when parts of the cadaver are quite fresh, is a chemical detection of hydrogen sulphide poisoning that is free from objection possible, for this gas is always formed when putrefaction sets in. Suspend in the containers over the parts of the cadaver a strip of paper moistened with lead acetate solution, or pass a stream of carbon dioxide washed with water through the objects tested and note whether the gas that issues colors "lead paper" brownish black as hydrogen sulphide does.

[1] K. B. Lehmann: Archiv. für Hygiene 14 (1892), 135.

A spectroscopic examination of the blood of a person poisoned by hydrogen sulphide is often of value. For this purpose examine in the spectroscope a 2.5–3 per cent. aqueous solution of blood taken from the brain (Harnack). If the blood contains hydrogen sulphide, a rather sharply outlined absorption-band between C and D appears together with the broad band of reduced haemoglobin. In case of doubt, saturate the given blood with hydrogen sulphide washed through water, make a solution of the above strength, and compare it with the blood in question. If a blood examination immediately follows death from hydrogen sulphide, or from inhalation of air containing less of the gas, the result of this test is usually negative. According to the thickness of the layer, blood containing hydrogen sulphide has a dirty green to dirty brown color.

Detection in Urine

To arrive at a definite conclusion from a urine examination as to poisoning by hydrogen sulphide, the urine should be drawn as freshly as possible, for hydrogen sulphide is set free as a result of putrefaction of certain organic sulphur compounds in every normal urine, such as oxyproteic acid, alloxyproteic acid and cystine. Wash a current of air by means of potassium hydroxide solution, pass it through the urine, and then through a narrow glass tube, suspending at the exit a strip of moist "lead paper." The urine may also be examined by the Caro-Fischer methylene blue test (see below under detection of hydrogen sulphide in water). For this purpose add to the urine 5 drops of hydrochloric acid, 1–2 drops of ferric chloride solution and a few particles of para-amino-dimethyl-aniline hydrochloride. A blue coloration of the urine mixture shows hydrogen sulphide.

Detection in Air

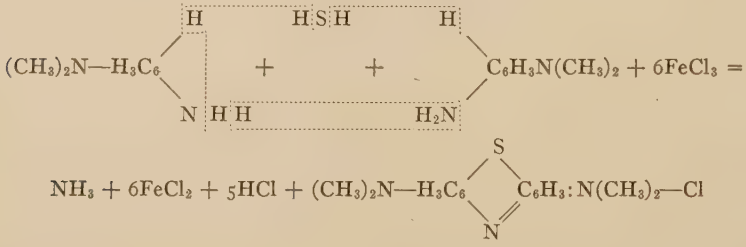
More than traces of hydrogen sulphide may be recognized by its characteristic odor. Also silver and copper objects turn brown or black in an atmosphere containing this gas. "Lead paper" behaves in the same way. A strip of paper saturated with a freshly prepared solution of sodium nitroprusside containing a little ammonia turns violet in presence of hydrogen sulphide. The latter test may also be made by passing the suspected air through dilute ammonia solution containing a few drops of dilute sodium nitroprusside solution. A violet coloration shows hydrogen sulphide. Another good test consists in passing the air through a pure aqueous solution of arsenious oxide (As_2O_3). Hydrogen sulphide colors this intensely yellow from formation of colloidal arsenic sulphide. The latter upon addition of a few drops of hydrochloric acid is precipitated as yellow arsenious sulphide (As_2S_3). The hydrosol of arsenious sulphide is converted into its gel.

Detection in Water

In case presence of hydrogen sulphide in the water cannot be recognized by odor, test with alkaline lead solution (lead acetate solution containing excess of sodium hydroxide solution). A brown coloration, or brownish black precipitate, shows that it is present. A violet coloration in dilute ammonia solution containing a few drops of aqueous sodium nitroprusside solution (1:100) is another

test that may be used. The Caro-Fischer reagent[1] is very sensitive to hydrogen sulphide. To detect very small quantities of this gas in aqueous solution, add first about $\frac{1}{50}$ of the volume of the solution of fuming hydrochloric acid. Then drop in a few small particles of para-amino-dimethylaniline sulphate. As soon as these have dissolved, add 1–2 drops of ferric chloride solution. In presence of hydrogen sulphide the mixture after some time takes on a pure blue color.

This test depends upon formation of methylene blue when an acid solution of para-amino-dimethylaniline is treated with hydrogen sulphide and a little ferric chloride:

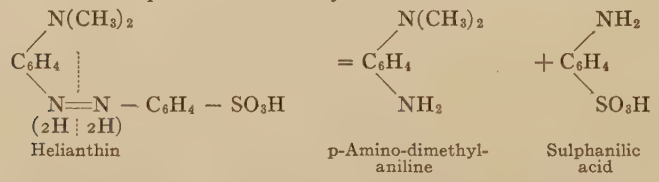


$$NH_3 + 6FeCl_2 + 5HCl + (CH_3)_2N{-}H_3C_6{-}S{-}C_6H_3{:}N(CH_3)_2{-}Cl$$

A large excess of hydrochloric acid is used to prevent formation of a red dyestuff when ferric chloride acts upon para-amino-dimethylaniline alone in neutral or faintly acid solution. Formation of methylene blue, according to E. Fischer, is the most sensitive and positive test for hydrogen sulphide in neutral or acid aqueous solution as the following experiment shows:

In this test 1 liter of distilled water containing 0.09 mg. of hydrogen sulphide was taken. First 20 cc. of fuming hydrochloric acid (sp. gr. 1.19) were added, then a few particles (about 5 mg.) of para-amino-dimethylaniline sulphate, and finally 2 drops of dilute ferric chloride solution. The mixture was at room temperature. Formation of dyestuff began at once and after about $\frac{1}{2}$ hour reached a maximum. The solution had a strong pure blue color that held for a day.

Preparation of Amino-dimethylaniline.—According to E. Fischer, the indicator helianthin, or Orange III, para-dimethyl-amino-azo-benzene sulphonic acid, is a convenient starting-point in the preparation of this reagent. Like most azo-dyestuffs it is reduced by nascent hydrogen and is thereby broken up into sulphanilic acid and para-amino-dimethylaniline:



Helianthin — p-Amino-dimethyl-aniline — Sulphanilic acid

Reduction takes place upon heating with hydrochloric acid and zinc dust, or even more easily with ammonium sulphide. Pour about 5 parts of water and excess of ammonium sulphide, that is, 2–4 parts upon finely ground helianthin and shake the cold mixture frequently. The dyestuff dissolves completely.

[1] E. Fischer: Formation of Methylene Blue as a Test for Hydrogen Sulphide. Ber. d. Deutsch. chem. Ges. 16 (1883), 2234.

After 24 hours the orange color has disappeared and reduction is complete. If the above mixture is warmed for 10–15 minutes upon the water-bath, cleavage of helianthin takes place more rapidly and smoothly. After complete reduction, extract the solution of para-amino-dimethylaniline with ether. The ether extract also contains some ammonium sulphide which should be removed by agitation with a little lead white suspended in water. Then cautiously add to the ether solution an ether solution of concentrated sulphuric acid. Neutral sulphate of para-amino-dimethylaniline separates as a nearly colorless thick mass. Excess of sulphuric acid should be avoided, otherwise the badly crystallizing acid salt is formed. The precipitated salt separated from ether is warmed upon the water-bath with 4–5 parts of absolute alcohol until it has changed into fine white needes. These are filtered off when cool, washed with alcohol, pressed and dried at 100°. In a closed vessel this preparation remains practically unchanged. Its solution in water has a faint brown color and is well suited for the hydrogen sulphide test.

Quantitative Estimation of Hydrogen Sulphide in Air

(Lehmann)

This method is based upon the reaction taking place between iodine and hydrogen sulphide in aqueous solution:

$$H_2S + I_2 = 2HI + S$$

By means of an aspirator, draw 8–10 liters of air in about 40 minutes through 10 cc. of 0.01 n-iodine solution and then through another flask containing 10 cc. of 0.01 n-thiosulphate solution to catch any iodine carried over mechanically. At the end of the experiment mix the two 0.01 n-solutions, add starch solution as indicator, and titrate excess of thiosulphate solution with 0.01 n-iodine solution. The number of cc. of 0.01 n-iodine solution required corresponds to the quantity of iodine that reacted, according to the above equation, with hydrogen sulphide. 1000 cc. of 0.01 n-iodine solution correspond to $\frac{H_2S}{200} = \frac{34}{200} = 0.17$ gram of hydrogen sulphide and 1 cc. of 0.01 n-iodine solution to 0.0017 gram or 0.17 mg. of hydrogen sulphide.

Sulphur Dioxide and Sulphurous Acid

Inhalation of sulphur dioxide produces effects both local and remote, as its aqueous solution does when taken internally. Because of easy conversion into sulphuric acid, like that acid it has a strong local corrosive action. Remote action is due to acidification and decomposition of the blood. These effects are also produced by acid sulphites, whereas local action of neutral salts is milder and less irritating, the latter having rather a paralyzing action upon the nervous system (R. Kobert). Lehmann[1] found that inhalation of air containing in 1000 volumes 0.006–0.010 volumes of sulphur dioxide causes nasal and bronchial irritation. It was unpleasant, though endurable for a short time, to remain in air containing 0.015–0.02 volumes, whereas 0.03 volumes rendered many persons, especially those who were sensitive, quite ill. There was stinging pain in the

[1] K. B. Lehmann: Archiv. für Hygiene 18 (1893), 180.

nose, sneezing and coughing. Kobert observed in a ligno-sulphite inhalatorium that individuals differed widely in sensitiveness to inhaled sulphur dioxide, habituation frequently taking place very quickly. Aqueous sulphurous acid and its salts may to some extent give rise to severe toxic effects. The gastric juice sets sulphur dioxide free from sulphites and a 0.5–1 per cent. solution of this gas may exert quite a poisonous action, manifesting itself in corrosions, blood decomposition, as shown by coagulation and formation of haematin with brown coloration, and finally paralysis of the nervous system.

Sulphurous acid or its salts should not be used to preserve foods, since even in small quantity they may cause injury to health. In experiments with neutral sodium sulphite, used as a preserving salt, Kionkas added only 0.2 per cent. to meat and produced severe blood decomposition in dogs by long continued feeding. Frequently beer and wine contain traces of sulphurous acid, since it is formed in many fermentations. In case of beer it may come from sulphured hops. In a great many wines Kerp[1] determined free sulphurous acid, in 75 per cent. of which he detected 1–10 mg. per liter and in 5 per cent. 11–20 mg. So it may be said that commercial wines in general do not contain more than 20 mg. of free sulphur dioxide in the liter. Kerp also detected in wine the presence of aldehyde sulphurous acid. In aqueous solution this acid undergoes hydrolytic cleavage into acetic aldehyde and sulphurous acid, depending upon concentration and temperature of solution. Dissociated sulphur dioxide can be titrated directly with iodine solution and is designated as "free sulphurous acid," expressed as SO_2. Hydrolytic cleavage of acetaldehyde-sulphurous acid is only slight even when the solution is highly diluted. For this reason free sulphurous acid is present in urine only in small quantity.

Detection and Estimation of Sulphurous Acid

(a) Add sulphur-free zinc and hydrochloric acid to the material and by means of lead paper test hydrogen for hydrogen sulphide. A positive test establishes presence of sulphurous acid. Zinc and hydrochloric acid should be tested alone to make sure that hydrogen sulphide does not come from reagents.

(b) Put in a capacious glass flask, connected on one side with a carbon dioxide generator and on the other side with a Liebig condenser, 200–300 cc. of wine together with 20 cc. of officinal phosphoric acid (containing 25 per cent. of H_3PO_4), and about 10 cc. of 0.1 n-iodine solution in the receiver. Pass a stream of carbon dioxide washed by water through the wine and after about 15 minutes begin distillation. Distil off about one-third of the wine. The distillate should contain excess of free iodine, otherwise more iodine solution must be added. Acidify with dilute hydrochloric acid, heat and precipitate sulphuric acid by barium chloride in the usual manner. The weight of barium sulphate multiplied by 0.274 gives the quantity of sulphur dioxide: $BaSO_4$ (233) : SO_2 (64) = 1 : x = 0.274.

Detection of Sulphur Dioxide in Air

The sharp smell of sulphur dioxide makes possible its detection in air when more than traces are present. Even traces of this gas may be detected in air

[1] W. Kerp: Sulphurous Acid in Wine. Arbeiten aus dem Kaiserl. Gesundheitsamt 21 (1904), 141, 156, 180, 372.

with certainty by means of paper saturated with starch paste containing iodic acid. Liberation of iodine causes it to turn blue:

$$(\alpha)\quad HIO_3 + 3H_2SO_3 = HI + 3H_2SO_4,$$
$$(\beta)\quad HIO_3 + 5HI = 3I_2 + 3H_2O.$$

The suspected air may also be passed through pure water. If sulphurous acid is present, the water will then bleach blue litmus paper and, after addition of chlorine water, give a precipitate with barium chloride showing presence of sulphuric acid.

Detection of Sulphurous Acid as Such in the Cadaver

After fatal poisoning by sulphurous acid, or sulphite, it is hardly ever possible to detect sulphurous acid in the cadaver because of the ease with which it is oxidized. Sometimes an abnormally high percentage of sulphate in stomach contents and blood makes possible the conclusion that poisoning by sulphur dioxide has taken place. The blood also shows change of color. In case of animals poisoned by free sulphurous acid arterial blood has a strikingly dark color and in some instances is said even to react acid (Ogata). On the other hand, neutral sulphites cause blood to take on a brick-red color.

Official Directions (German) for Detection and Estimation of Sulphurous Acid and Salts and of Hyposulphites in Meat

Mix upon the bottom of a 100 cc. Erlenmeyer flask as quickly as possible 30 grams of finely comminuted meat and 5 cc. of 25 per cent. phosphoric acid. Then at once stopper the flask. The end of the cork projecting into the flask has a slit holding a strip of potassium iodate-starch paper moistened for about 1 cm. on its lower end so that it hangs about 1 cm. above the centre of the mass of meat. The solution for this test-paper is made by dissolving 0.1 gram of potassium iodate (KIO_3) and 1 gram of soluble starch in 100 cc. of water.

If within 10 minutes the strip shows no blue color, which usually appears at the juncture of the moist and dry paper, loosen the cork slightly and set the flask upon the water-bath. If within 10 minutes the strip fails to show either a fugitive or a permanent blue color, then again stopper the flask tightly and allow it to cool in air. Finally, if within 30 minutes the strip shows no blue color, the meat is regarded as free from sulphurous acid. On the other hand, if the strip turns blue, a final test for sulphurous acid should be made by the following method:

(a) Mix 30 grams of finely comminuted meat in a 500 cc. distilling flask with 200 cc. of boiled water, adding sodium carbonate solution until faintly alkaline. Allow to stand 1 hour and then close the flask with a two-holed stopper through which two glass tubes enter the flask. The first tube extends to the bottom of the flask and the second only into the neck. The latter connects with a Liebig condenser having at the other end an air-tight connection by means of a bored stopper with a so-called Péligot tube.

Pass carbon dioxide (water-washed) through the tube leading to the bottom of the flask until air is completely expelled from the apparatus. Then put 50 cc. of iodine solution into the Péligot tube, made by dissolving 5 grams of pure

iodine and 7.5 grams of potassium iodide in a liter of water. This solution should be free from sulphate. Raise the stopper of the distilling flask and, without interrupting the stream of carbon dioxide, run in 10 cc. of 25 per cent. aqueous solution of phosphoric acid. At once stopper the flask, carefully heat the contents, and distil off half the aqueous solution all the while keeping up the stream of carbon dioxide. Now transfer the iodine solution, which should have a brown color, to a beaker, thoroughly rinse the Péligot tube with water, add some hydrochloric acid, heat for a short time, and precipitate with barium chloride solution (1 part of crystallized $BaCl_2.2H_2O$ in 10 parts of distilled water) sulphuric acid formed by oxidation of sulphurous acid. Usually it is not absolutely necessary to weigh barium sulphate. If, however, for a special reason the precipitate should be weighed, allow it to settle and make sure that all sulphuric acid is precipitated by adding a drop of barium chloride solution on top of the clear liquid. Again heat to boiling, keep warm and allow to stand for 6 hours, pour the clear liquid through a paper of known ash-content, repeatedly wash the precipitate in the beaker with hot water, each time allowing the liquid to settle until clear and pouring it through the filter. Finally bring the precipitate upon the filter and wash with hot water until the filtrate no longer shows cloudiness with silver nitrate solution. Then dry filter and precipitate, ash in a weighed platinum crucible and ignite. Moisten the contents of the crucible with a little sulphuric acid, expel the latter, ignite gently, allow to cool in desiccator and weigh.

If this test gives a positive result, the meat is considered as having been treated with sulphurous acid, sulphites, or hyposulphites. If necessary to establish whether sulphurous acid came from hyposulphites, the following method may be employed:

(b) Repeatedly stir and lixiviate in a beaker 50 grams of comminuted meat with 200 cc. of water and enough sodium carbonate solution to render faintly alkaline. Press out the meat and filter the extract. Acidify strongly with hydrochloric acid, add 5 grams of pure sodium chloride and heat. Filter off the precipitate obtained and wash until wash-water contains neither sulphurous nor sulphuric acid. Then dissolve the precipitate in 25 cc. of 5 per cent. sodium hydroxide solution, add 50 cc. of saturated bromine water and heat to boiling. Now acidify with hydrochloric acid and filter. The entirely clear filtrate gives in presence of hyposulphites in meat an immediate precipitate of barium sulphate upon addition of barium chloride solution.

Boric Acid

For a long time boric acid was regarded as a substance having no action whatever upon the human organism. The belief was general that even after prolonged use the body suffered no injury. But it is now known that boric acid, however used, more especially after injection of its solutions into body cavities, due to absorption may give rise to severe poisoning that may terminate fatally. When food containing small quantities of boric acid as a preservative has been used for a long time, injurious effects have been observed. Frequent use of milk containing boric acid, for example, has been found to cause illness. Nausea and vomiting followed the ingestion of 8 grams of boric acid within an hour. For several hours, pressure and a feeling of fullness in the stomach persisted. Furthermore external use of boric acid for antiseptic irrigation has been the cause

of injurious effects that have even terminated fatally. Lewin thinks that individualism enters in, in so far as it influences elimination of boric acid, making it slow or rapid. In general boric acid is eliminated slowly by way of the saliva and urine. Any interference, however slight, with elimination of boric acid has brought about its accumulation in the body and given rise to unpleasant effects. Schlenker[1] has shown from metabolism experiments on man that daily use of 1.5–3 grams of boric acid has a strong influence upon the absorptive power of the intestinal tract and causes excretion of considerable intestinal mucus. Many investigators who have studied the question of harm done to the human body by boric acid and borax have concluded from their experiments that even small quantities of these two substances may cause irritation of the gastro-intestinal tract. This may manifest itself in vomiting, diarrhoea, impaired digestion of food and consequent emaciation. Skin eruptions may also appear, as eczema, petechiae,[2] erythema and oedema. Boric acid is deposited in the organs, that is, a part of the acid is stored up and slowly eliminated by the salivary glands, gastric mucosa, milk glands, but mainly by the blood through the kidneys. Nephritis is said to arise possibly from this cause. Disturbances of vision have also been observed (Kobert).

As the result of experiments in which borax was fed to dogs, boric acid was detected in the liver, bile, kidneys, blood and lungs. After subcutaneous injection it was found also in the gastro-intestinal mucosa.

Detection of Boric Acid

Flame Test.—In examining urine, render it strongly alkaline with sodium carbonate, evaporate to dryness upon the water-bath, mix the residue well with concentrated sulphuric acid (4–5 cc.), add alcohol (preferably methyl alcohol), stir again and ignite the alcohol. In presence of boric acid, the yellow flame will have a green mantle which may be seen best by stirring the mixture with a glass rod. The green color of the flame may also be seen to advantage in a dark room.

Turmeric Test.—Evaporate the urine, or comminuted organ, to dryness with sodium carbonate and potassium nitrate. Ignite the residue but not too strongly nor too long. Dip a strip of turmeric paper in the solution of the residue after acidification with hydrochloric acid. In presence of boric acid the paper has a red-brown color which comes out especially when the paper is dried. Then if moistened with potassium hydroxide solution the color of the paper changes to dark greenish blue or blackish blue.

[1] G. H. Schlenker: Use of Boric Acid as a Food-Preservative. Dissertation, München 1883.

[2] Petechiae are small, usually roundish hemorrhages about the size of a pinhead in the corium. They differ from erythema in not losing their color upon pressure.

Quantitative Estimation of Boric Acid

1. **Jörgensen's[1] Method.**—Boric acid in aqueous solution titrated with sodium hydroxide solution and phenophthalein gives a red color after addition of a slight amount of alkali, approximately 1 mol. of sodium hydroxide to 6 mols. of boric acid. In presence of considerable glycerol, or mannitol, boric acid behaves like a monobasic acid, that is, a solution of 1 mol. of boric acid with phenolphthalein turns red after addition of exactly 1 mol. of sodium hydroxide solution. For example, dissolve 0.4–0.5 gram of boric acid in as little water as possible, add 50 grams of neutral glycerol and phenolphthalein solution, and titrate with 1.0 or 0.1 n-sodium hydroxide solution to red color. The equivalent weight of boric acid = mol. wt. = H_3BO_3 = 61. So 1 cc. of 1.0 n-sodium hydroxide solution under the above conditions neutralizes 0.061 gram of boric acid.

Application of Method to Estimation of Boric Acid in Meat, Milk, Urine, Etc.—Add sodium hydroxide solution to 50 grams of meat, 100 cc. of milk or urine, until strongly alkaline. Mix thoroughly, dry, and ash. Dissolve the residue in dilute sulphuric acid, warming to expel carbon dioxide. Cool, dilute to 50 cc., add phenolphthalein and, disregarding any precipitate (H_3BO_3?), exactly neutralize with 1.0 n-sodium hydroxide solution. Then add 50 cc. of neutral glycerol and titrate to the end with 0.1 n-sodium hydroxide solution. Under as nearly as possible the same experimental conditions determine the titer of this sodium hydroxide solution, using boric acid solution (5 cc.) of known content, for example, one containing 0.1 gram of pure crystallized boric acid in 50 cc. Calculate the titer on the basis of the number of cc. of 0.1 n-sodium hydroxide solution used after addition of glycerol. Addition of ethyl alcohol at the end of the titration of boric acid is said to make the end-reaction sharper.

2. **Partheil and Rose[2] Method.**—Boric acid distils rather easily with steam, also with the vapor of methyl or ethyl alcohol! Commercial ether distilled with boric acid carries over small quantities of the acid. But no boric acid volatilizes when ether is sucked

[1] G. Jörgensen: Volumetric Estimation of Boric Acid. Zeitschr. f. angew. Chemie 1897, 5.

[2] A. Partheil and J. A. Rose: Direct Gravimetric Estimation of Boric Acid. Ber. d. Deutsch. chem. Ges. 34 (1901), 3611.

off over boric acid *in vacuo* over sulphuric acid. Boric acid dissolves but slightly in pure absolutely dry ether; it is considerably more soluble in pure ether saturated with water, 100 grams of the latter solvent dissolving 0.188 gram of boric acid. Based upon these observations Partheil and Rose have devised a method of estimating boric acid gravimetrically by means of the extraction apparatus[1] shown in Fig. 25.

FIG. 25.—Partheil-Rose extractor.

Procedure.—Pour the boric acid solution acidified with hydrochloric acid into the spirally wound tube until at the highest it reaches only to the bulb. Then carefully add enough freshly rectified ether nearly to fill the bulb. Put 20 cc. of the same ether in the flask weighed with a piece of pumice-stone used to prevent bumping. Then heat upon the water-bath and have the ether in lively ebullition so that it comes from the condenser not in drops but in a continuous stream. In this manner extract for at least 18 hours. Then put on a second weighed flask to make sure after about 2 hours extraction that exhaustion is complete. In each case the flask with the ether solution is put into a vacuum desiccator (Fig. 13, page 96) over sulphuric acid, the ether sucked off, and residual boric acid dried to constant weight and weighed as H_3BO_3. Using this method, Partheil and Rose obtained 0.3105 and 0.31025 gram of boric acid from an aqueous solution containing 0.31015 gram of the acid. A borax solution gave 0.12425 instead of 0.12408 gram of boric acid.

Notes.—Boric acid to be extracted with ether should not contain free sulphuric phosphoric or nitric acid, or appreciable quantities of iron. The extractor recommended by Partheil and Rose may be used to advantage in extracting alkaloids from aqueous solutions rendered alkaline with sodium hydroxide solution.

This method is applicable to determination of boric acid in margarines, for example. Melt 50 grams of the sample with 20 cc. of water and allow the fat to solidify again completely. Perforate the crust and decant the aqueous solution Repeat this operation 3–4 times. Render the combined aqueous extracts alkaline with sodium hydroxide solution, evaporate, ash, and extract the solution of the ash in hydrochloric acid as above. Preceding extraction with ether, remove sul-

[1] This extractor is supplied by the Firm of C. Gerhardt, Bonn.

phuric acid with barium chloride, phosphoric acid with ferric chloride, as well as nitric acid, from the alkaline residue left upon evaporation. Directions as follows are given[1] for milk and meat: Ash 50 cc. of milk, or 20 grams of chopped meat, with 1 gram of sodium carbonate. Add a few drops of ferric chloride solution to the faintly acidified filtrate of the ash, to remove completely PO_4''', precipitate excess of iron with sodium hydroxide solution, filter, acidify the filtrate with hydrochloric acid, evaporate to 15 cc., and extract in the extractor with ether. Proceed with the examination of urine in the same manner.

Official Directions (German) for Detection of Boric Acid and Salts in Meats

Make a uniform mixture in a beaker of 50 grams of finely comminuted meat with a mixture of 50 cc. of water and 0.2 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.). Allow the mass to stand in the beaker for half an hour covered with a watch-glass, stirring occasionally, and heat for half an hour in a boiling water-bath. Throw the contents of the beaker while warm upon a piece of cheese-cloth, press the meat well, and pour the liquid through a moist filter. Add phenolphthalein to the filtrate, render slightly alkaline with 0.1 n-sodium hydroxide solution, and concentrate to 25 cc. Acidify 5 cc. of this liquid with 0.5 cc. of hydrochloric acid (sp. gr. 1.124) filter, and test for boric acid with turmeric paper (see Preparation of Reagents, page 644). To do this, moisten a strip of smooth turmeric paper 8 cm. long and 1 cm. wide for about half its length with the acidified solution, drying at 60–70° upon a watch-glass about 10 cm. in diameter. If the turmeric paper shows no visible change in its original yellow color, the meat contains no boric acid. But, on the other hand, if a reddish or orange-red color appears, touch the paper where it has changed color with a 2 per cent. aqueous solution of sodium carbonate. If this produces a red-brown spot not differing in color from the red-brown spot developed by sodium carbonate solution alone upon pure tumeric paper, or a red-violet color, the meat also contains no boric acid. But if the sodium carbonate solution produces a blue spot, presence of boric acid is shown. In case of blue-violet colorations and in doubtful cases, the result of the flame test is conclusive. This should be made in the following manner:

Evaporate to dryness and ash in a platinum dish 5 cc. of the residual alkaline liquid. To prepare the ash, lixiviate the carbonized substance with about 20 cc. of hot water. Completely ash the carbon over a small flame, add the liquid from lixiviation, and bring to dryness first upon the water-bath and then at about 120°. Carefully triturate the light ash thus obtained with a cold mixture of 5 cc. of methyl alcohol and 0.5 cc. of concentrated sulphuric acid, using 5 cc. more methyl alcohol to transfer to a 100 cc. Erlenmeyer flask. Frequently shake the closed flask and allow to stand for half an hour. Then completely distil off methyl alcohol from a water-bath at 80–85°. Transfer the distillate to a glass vessel about 6 cm. high having a volume of 40 cc. Close this by means of a two-hole stopper through which two glass tubes pass, one extending to the bottom the other only as far as the neck. The outer end of the latter tube is reduced in bore and provided with a perforated platinum tip that may be made from foil. Pass through the liquid a stream of dry hydrogen so that the ignited flame is about

[1] A. Partheil and J. Rose: Direct Gravimetric Estimation of Boric Acid in Foodstuffs. Zeitschr. f. d. Untersuchung von Nahrungs-Genussmitteln 5 (1902), 1049.

2–3 cm. long. If the flame observed in diffused daylight has a green color, the meat contains boric acid. Meat in which boric acid is detected after carrying out these directions is to be regarded as having been treated with boric acid or its salts.

Chloric Acid and Chlorates

Large doses (4–10 grams) of potassium chlorate, $KClO_3$, are decidedly toxic. In the case of adults 15–30 grams of this salt are usually fatal. In many instances death does not ensue until several days later. During the first stage of intoxication, alteration in the shape of red corpuscles and conversion of oxyhaemoglobin in the intact corpuscles into brown methaemoglobin take place. Then red blood-corpuscles, at least in a case of severe poisoning, change their form, becoming shriveled; and undergoing decomposition. Toxicologists (see R. Kobert, intoxikationen) ascribe change of blood-pigment and red blood-corpuscles to specific salt action possessed in high degree by potassium chlorate. This explanation also accounts for salt diuresis, appearing at the beginning of potassium chlorate poisoning, whereby the blood is much thickened. But most notable is the high alkalinity of the urine, resulting in decreased alkalinity of the blood plasma. In severe chlorate poisoning so much oxyhaemoglobin is changed to methaemoglobin that the amount of oxygen in the blood may drop to 1 per cent. As a result, human beings or animals thus poisoned may become asphyxiated from lack of oxygen. Potassium chlorate through the action of potassium weakens the heart. In chlorate poisoning the blood has a characteristic chocolate-brown color.

Potassium chlorate taken by the mouth is rapidly absorbed from the stomach and soon eliminated by the kidneys. After administration of 0.1 gram of potassium chlorate, chloric acid appears in the urine in an hour. Most of the potassium chlorate passes into the urine unchanged, only a little of the salt being reduced to potassium chloride. During chlorate poisoning, the urine is usually very dark, even black, and may contain haemoglobin and methaemoglobin. It is frequently opaque and strongly alkaline. Upon long standing a dark brown sediment gradually deposits. The urine also contains considerable albumin.

In suspected chlorate poisoning, the urine should if possible receive thorough chemical and microscopical examination. An anuria lasting several days may precede death and render an examination of the urine quite impossible.

Detection of Chloric Acid

To isolate potassium chlorate from organic material, use a dialyzer that should be as flat as possible, because the thinner the layer in the inner container and the larger the volume of water in the outer vessel, the more rapid the diffusion. Place the material, such as parts of organs and stomach or intestinal contents, in the inner container of a flat dialyzer and pure water in the outer vessel. Allow dialysis to take place 5–6 hours without changing the water in

the outer vessel. Then evaporate the dialysate (contents of the outer vessel) to dryness in a porcelain dish upon the water-bath. Dissolve the residue in a little water and examine the filtered solution for chloric acid (ClO_3' ion) as follows:

1. **Indigo Test.**—Add dilute sulphuric acid and a few drops of indigo solution, until the color is distinctly blue. Then introduce sulphurous acid drop by drop. If chloric acid is present, the blue color changes to yellow or greenish yellow. This is a delicate test for chloric acid, given even by 0.01 gram of potassium chlorate.

2. **Silver Nitrate Test.**—Add silver nitrate solution in excess. If there is a precipitate (AgCl), filter, add a few drops of sulphurous acid to the clear filtrate, as well as an excess of dilute nitric acid, and boil. A chlorate will cause the precipitation of more silver chloride. Silver chloride differs from silver sulphite in being insoluble in hot dilute nitric acid. Sulphurous acid reduces silver chlorate to chloride:

$$AgClO_3 + 3H_2SO_3 = AgCl + 3H_2SO_4$$

3. **Free Chlorine Test.**—A solution containing a chlorate, heated with concentrated hydrochloric acid, gives free chlorine. The gas passed into potassium iodide solution liberates iodine. Shake the solution with chloroform which dissolves iodine with violet color. This test indicates chloric acid only in absence of substances like chromic acid and dichromates which also give chlorine with hydrochloric acid.

Quantitative Estimation of Chloric Acid

To estimate potassium chlorate quantitatively in urine, dialysates and other liquids, reduce with zinc dust (I), or employ Scholtz's method (II[1]).

I. **Zinc Dust Method.**—Divide the solution into two equal parts. Determine chloride gravimetrically in one portion by precipitating and weighing AgCl, or volumetrically by titrating according to Volhard's method.

In the second portion determine chloride and chlorate together. Add 5–10 grams of zinc dust and a little dilute sulphuric acid, or better acetic acid, and heat the mixture 0.5–1 hour upon a boiling water-bath. Filter and wash the residue with boiling water. Acid-

[1] M. Scholtz: Volumetric Estimation of Chlorates and Bromates. Archiv d. Pharmaz. 243 (1905), 353.

ify the filtrate with dilute nitric acid and determine Cl′-ion as above. More chlorine appears in the second than in the first determination. Calculate percentage of potassium chlorate from the difference between the two chlorine determinations. One molecule of $KClO_3$ upon reduction yields 1 molecule of KCl and therefore 1 molecule of AgCl (143.35) corresponds to 1 molecule of $KClO_3$ (122.45).

Zinc dust together with acetic acid reduces potassium chlorate to potassium chloride:

$$KClO_3 + 3Zn + 6CH_3-COOH = KCl + 3(CH_3-COO)_2Zn + 3H_2O$$

II. Method of Scholtz.—This method makes use of the reducing action of nitrous acid upon chloric acid:

$$HClO_3 + 3HNO_2 = HCl + 3HNO_3$$

Add to the solution, for example, the evaporated dialysate, 10 cc. of nitric acid (sp. gr. 1.2 = 32 per cent.) and 10 cc. of 10 per cent. sodium nitrite solution. Allow the mixture to stand for 15 minutes at room temperature. Then add 30–50 cc. of 0.1 n-silver nitrate solution and 5 cc. of saturated iron alum solution. Titrate excess of silver with 0.1 n-ammonium sulphocyanate solution. 1000 cc. of 0.1 n-$AgNO_3$ = 0.1 $KClO_3$ gram = 12.245 grams of $KClO_3$.

The slight excess of nitrous acid has no effect upon the delicacy of the reaction. Liquids like dialysates of stomach-contents and organs always contain chloride. In that case first determine the quantity of chloride by means of 0.1 n-silver nitrate solution, using potassium chromate as indicator. Nitrous acid does not reduce an aqueous solution of potassium perchlorate, $KClO_4$, which may possibly be present.

Hildebrandt[1] has adapted Scholtz's method to the examination of urine. First completely precipitate chloride in a measured volume of urine with silver nitrate in presence of nitric acid. Add sodium nitrite solution to the clear, chloride-free filtrate, as well as more silver nitrate solution, until a precipitate no longer appears. Determine as usual the weight of silver chloride obtained. The corresponding quantity of potassium chlorate is obtained by multiplying this weight by 0.854 : $KClO_3$: AgCl = 122.45 : 143.33 = 0.854.

In the case of urine a larger quantity of nitrous acid is decomposed by urea:

$$CO(NH_2)_2 + 2HNO_2 = CO_2 + 2N^2 + 2H_2O$$

Consequently do not use too little sodium nitrite.

[1] H. Hildebrandt: Detection of Chlorates in Urine. Vierteljahrsschr. f. gerichtl. Medizin u. öff. Sanitätswesen 32 (1906), 81.

Behavior of Potassium Chlorate in Putrefaction

C. Bischoff states that potassium chlorate, mixed with moist, organic substances, especially blood, is very soon reduced to chloride! Bischoff describes several cases, in which poisoning by potassium chlorate had undoubtedly occurred, and yet chloric acid could not be detected chemically in parts of the cadaver. In an experiment, 100 grams of blood, 0.5 gram of potassium chlorate and 100 grams of water were allowed to stand for 5 days at room temperature. Not a trace of chloric acid could be detected in the dialysate. Bischoff concludes from this experiment that potassium chlorate, mixed with moist organic substances, especially with blood, is soon reduced. Consequently, chloric acid may not be detected, even in cases of rapidly fatal poisoning by potassium chlorate.

Official Directions (German) for Detection of Chlorates in Meat

Allow 30 grams of finely comminuted meat to stand for 1 hour in the cold with 100 cc. of water and then heat to boiling. Filter when cold and add silver nitrate solution in excess to the filtrate. Add 1 cc. of 10 per cent. sodium sulphite solution and 1 cc. of concentrated nitric acid to 25 cc. of the clear filtrate from the silver precipitate and then heat to boiling. If a precipitate appears, insoluble in more hot water and consisting of silver chloride, chlorate is present. Meat examined according to these directions and giving a positive test is regarded as having been treated with chlorates.

Explanation.—Chlorate added to meat as a preservative gives with silver nitrate silver chlorate, $AgClO_3$, which is easily soluble in water. Free sulphurous acid formed by the action of nitric acid upon sodium sulphite, reduces silver chlorate to chloride:

(a) $Na_2SO_3 + 2HNO_3 = 2NaNO_3 + H_2SO_3(SO_2 + H_2O)$
(b) $AgClO_3 + 3H_2SO_3 = AgCl + 3H_2SO_4$

To prove conclusively that the precipitate is silver chloride, collect it upon a filter, wash and dissolve in ammonia. Acidification of this solution with Cl′-free nitric acid will again precipitate silver chloride white and curdy.

Oxalic Acid[1]

Oxalic acid and its salts, for example, salt of sorrel, are quite toxic and rapid in action. Administration of oxalic acid has terminated fatally in the case of adults in a few minutes. Oxalic acid is very abundant in the plant kingdom in the form of its acid potassium salt, KHC_2O_4, and calcium salt. Sorrel, wood-sorrel, and rhubarb are especially rich in salts of oxalic acid. Hence this acid may find access to the body through food and drugs of vegetable origin. Moreover, oxalic acid in small quantity is a normal constituent of human urine, 2–10 mg. being excreted in the course of a day. Con-

[1] For reasons of convenience oxalic acid is included among inorganic substances in connection with inorganic acids.

sequently in examining parts of organs, stomach-contents, urine, and other materials it is often necessary to supplement a positive qualitative test by a quantitative estimation of oxalic acid.

Toxic Action.—An important difference between mineral acids and oxalic acid is the toxicity of the salts of the latter. Not only do free oxalic acid and its acid potassium salt, salt of sorrel, show poisonous properties but even very dilute solutions of neutral sodium oxalate, $Na_2C_2O_4$, act in the same way. Therefore in oxalic acid poisoning it is necessary to distinguish between local corrosion, occurring at the point of application and also in part upon elimination, and remote action due to absorption. Local action at the point of application is corrosive like that of all acids. Local action at the place of elimination depends upon formation and insolubility of calcium oxalate. On account of the ease with which the organism takes up oxalic acid and its alkali salts, action of the absorbed poison is rapid. The effects caused by its presence may be ascribed to the fact that this acid removes in part from organs, as the heart, and from body fluids (blood) calcium they require for their life processes, converting it in part into insoluble calcium oxalate. Oxalates diminish coagulating power as well as alkalinity of the blood. On the other hand, they increase the quantity of sugar in the blood. In oxalic acid poisoning there is a depression of the entire metabolism. This is also the case as regards taking up oxygen and giving off carbon dioide. The body temperature falls as processes of metabolism are retarded. Owing to withdrawal of calcium from the heart, that organ is weakened and finally paralyzed. Local action upon the kidneys is due to clogging of the injured urinary tubules by deposits of calcium oxalate. Flow of urine may wholly cease in consequence of total impairment of the urinary tubules and death may ensue from anuria and uraemia. Fatal poisonings from large doses of oxalic acid are usually of short duration. R. Kobert (Intoxikationen) describes a case where death occurred within 10 minutes.

Bischoff[1] has made statements with regard to distribution of oxalic acid in different organs of persons poisoned by this substance. In a case that terminated fatally in less than 15 minutes the quantity of oxalic acid in each organ was determined separately and found to be:

Weight	Organ	Oxalic Acid
2240 grams	Stomach, oesophagus, intestine and contents	2.28 grams
770 grams	Liver	0.285 gram
290 grams	Kidneys	0.0145 gram
180 grams	Blood from the heart	0.0435 gram
40 grams	Urine	0.0076 gram

The quantity of oxalic acid in the liver is noticeably large. The kidneys and urine contain only a little of the poison, owing to the short duration of life after poisoning. A striking thing about the urine excreted during oxalic acid poisoning is the abundant deposition of crystallized calcium oxalate.

[1] C. Bischoff: Distribution of Poisons in the Human Organism in Poisoning Cases. Ber. d. Deutsch. chem. Ges. 16 (1883), 1337.

Detection of Oxalic Acid

To detect oxalate without discriminating between free acid, acid potassium salt, or calcium oxalate, employ the following method:

Add to the finely divided material 3–4 volumes of alcohol and acidify with dilute hydrochloric acid. Stir frequently and allow the mixture to digest 1–2 hours in the cold. Then filter through creased paper moistened with alcohol and wash the residue with alcohol. To prevent formation of ethyl oxalate during evaporation, add about 10 cc. of water to the total filtrate. Evaporate upon the water-bath until all alcohol is expelled. Pass the aqueous residue through a small filter. Extract the filtrate in a separating funnel 3–4 times with 50–60 cc. portions of ether. Allow the total ether extract to stand for some time in a dry flask, then pass through a dry filter and distil. Dissolve the residue in 2–3 cc. of water and pass the solution, if necessary, through a moist filter. Add ammonium hydroxide solution until alkaline and then saturated calcium sulphate solution. If a precipitate appears, acidify with acetic acid and allow solution and precipitate to stand over night in a covered beaker. If there is still a crystalline precipitate, it can be only calcium oxalate. A microscopic examination of this precipitate is advisable. Calcium oxalate forms characteristic octahedrons having the so-called envelope-shape (Fig. 26). But other crystal forms may also appear. When thoroughly washed, calcium oxalate may be converted by ignition into calcium oxide that may be weighed.

FIG. 26.—Calcium oxalate crystals.

Calculation.—CaO (56):$H_2C_2O_4.2H_2O$ (126) = Wt. CaO found: x. Since the quotient 126:56 = 2.25, multiply the weight of calcium oxide found by 2.25 to get the corresponding amount of crystallized oxalic acid. Or dissolve the washed calcium oxide upon the filter with hot dilute sulphuric acid, warm the solution to about 80°, and titrate oxalic acid with approximately 0.1 n-potassium permanganate solution standardized against pure crystallized oxalic acid: 1000 cc. of 0.1 n-$KMnO_4$ solution = 6.3 grams of oxalic acid, $H_2C_2O_4.2H_2O$.

Nitrous Gases and Nitrites

So-called nitrous gases, or vapors, appearing as red fumes from the action of concentrated nitric acid upon many substances, especially those of organic nature, really consist of nitrogen dioxide, NO_2. Fuming nitric acid also contains this gas. Severe poisonings, some terminating fatally, due to inhalation of too large quantities of these nitrous gases, have been of frequent occurrence in factories making nitric acid, oxalic acid, nitrobenzene and picric acid. Toxic effects manifest themselves in difficult breathing, oppression of the chest, coughing, dizziness, cyanosis and bronchitis. During the early stages of intoxication vomiting of lemon-yellow masses, diarrhoea and collapse may also appear. In many instances the poisoning takes an entirely different course. After having breathed the poisonous vapors, the individual at first has almost no distress and even for an hour can continue working in rooms containing nitrogen dioxide. Then as a rule he experiences a feeling of thirst and need of fresh air. Usually after 6–8 hours without apparent outside cause marked difficulty in breathing and a feeling of uneasiness appear. He has burning thirst and thinks he is going to suffocate. The face is covered with cold sweat and, owing to formation of methaemoglobin, takes on a blue-gray color (cyanosis). The eyes are much swollen. At intervals lasting for several minutes convulsive attacks of coughing attended with vomiting occur. Within 40 hours death ensues from oedema and haemorrhage of the lungs. The first day the urine, always present in small quantity, may contain haematin and methaemoglobin, but not always, and an abundance of albumin.

Traces of nitrites occur normally in the human and mammalian organism, particularly in saliva, nasal mucus, bronchiae, parotis, lung tissue, kidneys, white brain-substance, lymph glands, and testicles (Stepanow[1]). This explains why all these animal fluids and organs in presence of carbon dioxide liberate iodine from potassium iodide (catarrh from iodine). It should also be noted that formation of nitrite is possible in the intestinal tract from the action of ever present reducing bacteria upon nitrates in food. Oxidation of ammonia also gives rise to nitrites. There are two classes of nitrite-forming bacteria, one causing formation by reduction of nitrates and the other by oxidation of ammonia (Maassen[2]).

Nitrites act upon blood-pigment, changing it, that is, oxyhaemoglobin, into methaemoglobin. They also have an irritating action upon mucous membranes, that is, upon those of the stomach and intestines. Finally they cause paralysis of the central nervous system, acting first upon the brain and then upon the spinal cord.

Sodium nitrite, however, seems to be rather mild in its action. Kobert cites the case of a patient who took hourly several doses of 0.5 gram of this salt without serious results. The only effects were nausea, diarrhoea, feeling of faintness, marked cyanosis, and increased diuresis.

[1] A. Stepanow: Detection of Nitrites in Many Organs. Archiv. f. exper. Path. u. Pharmakol. 47 (1902), 411.

[2] A. Maassen: Decomposition of Nitrates and Nitrites by Bacteria. Arbeiten aus d. Kaiserl. Gesundh.-Amte 18 (1901), 1.

Post-mortem Appearances.—Following inhalation of nitrous gases, the mucous membranes of the larynx, trachea and bronchiae are brownish in color. The blood in the various organs is of a black-brown color and deficient in alkalinity. The exudate upon the surface of an incision in the lungs is brownish.

Detection

To detect nitrites, dilute the material, such as stomach-contents, or vomitus, with water, acidify with acetic acid, and distil in a current of carbon dioxide, collecting the distillate under water. The following tests may be made:

1. Starch-iodide Test.—Add to a portion of the distillate starch-potassium iodide solution acidified with dilute sulphuric acid. Nitrous acid liberates iodine which produces a blue color with starch:

$$(a)\quad HONO + HI = NO + H_2O + I$$
$$(b)\quad NO_2 + 2HI = NO + H_2O + I_2$$

2. Azo-Dye Test.—Shake 10 cc. of distillate with 10 drops of naphthol reagent (see page 643) and 2 drops of concentrated hydrochloric acid. Then carefully add ammonia as an upper layer. In presence of nitrous acid a red ring appears at the zone of contact. If the tube is turned around, the entire liquid assumes a red color from formation of an oxy-azodyestuff.

3. Prussiate of Potash Test.—Even in very high dilution (1:600,000) potassium ferrocyanide solution in presence of acetic acid with nitrous acid turns yellow with oxidation to potassium ferricyanide and nitric oxide:

$$(N_2O_3.H_2O^1) + 2K_4[Fe(CN)_6] + 2H_4C_2O_2 = 2K_3[Fe(CN)_6] + 2KH_3C_2O_2 + 2H_2O + 2NO$$

4. Bismarck Brown Test.—Nitrous acid colors an aqueous solution of metaphenylene diamine (0.5:100) yellow-red to brown (formation of Bismarck Brown). A strip of filter paper moistened with the diamine solution may be used to detect nitrogen dioxide in the air. This test may be made more delicate, if a larger quantity of air is drawn through a solution of the diamine sulphate by an aspirator.

In addition to these tests, nitrous acid also gives the well-known nitric acid tests with ferrous sulphate, brucine and diphenylamine (see page 293). Nitrous acid differs from nitric acid in discharging the color of a solution of potassium permanganate to which sulphuric acid has been added.

Detection of Free Alkalies

Potassium, Sodium and Ammonium Hydroxides

In the detection of alkalies, the same general principles used in detecting mineral acids are applicable. Since potassium and sodium compounds are normal constituents of animal and plant organisms, and since ammonia is a decomposition product of nitrogenous organic matter, examination must always show that alkalies are in the free

[1] Instead of $2HNO_2$ is written $(N_2O_3.H_2O)$.

state, for they alone and their carbonic acid salts decompose and corrode animal tissues and not their neutral salts.

Poisonings due to caustic alkalies resemble those of corrosive acids. If taken internally, corrosive action gives rise to pain in the mouth, throat, oesophagus, stomach and abdomen. Mineral acid corrosions are dry and brittle ("solid mortification"), whereas those from caustic alkalies are soft and greasy. The alkali albuminates formed become gelatinous, swell and may partially dissolve in presence of much water. In forensic medicine the term "Colliquation" is used (softening, liquefaction). Destructive action of caustic alkalies extends deep and affects the parts around the corroded places. In caustic alkaline solutions gelatinous tissues, horny substances, hair and skin swell considerably and finally dissolve. The stomach in alkali poisoning is softened, corroded and decidedly bright red in color.

Ammonia

Free ammonia is usually recognized by odor. A piece of moist red litmus paper, held over the material, becomes blue. A paper moistened with mercurous nitrate solution is blackened.

Distillation.—If material is strongly alkaline, extract several times with absolute alcohol. Use a flask with glass stopper and distil the combined extracts. Collect the distillate in a little dilute hydrochloric acid and evaporate the solution to dryness upon the water-bath. Dissolve the residue in water and test the solution for ammonia, using Nessler's reagent, chloroplatinic acid, and sodium cobaltic nitrite, $Na_3[Co(NO_2)_6]$. The residue from distillation may be used in testing for fixed alkalies.

Fixed Alkalies

The residue from distillation may contain potassium and sodium hydroxides. If strongly alkaline, first add a few drops of phenolphthalein solution and then excess of barium chloride solution. The red color and alkaline reaction, if due to carbonates, disappear because two neutral salts are formed:

$$K_2CO_3 + BaCl_2 = BaCO_3 + 2KCl$$

But if fixed alkalies are present, alkaline reaction and red colors remain, for soluble barium hydroxide is formed:

$$2KOH + BaCl_2 = Ba(OH)_2 + 2KCl$$

The solution of the latter reddens phenolphthalein.

To distinguish potassium from sodium hydroxide, neutralize the remainder of the residue from distillation of alcohol with dilute hydrochloric acid and test the solution for potassium with chloroplatinic acid, $H_2[PtCl_6]$, and sodium cobaltic nitrite, $Na_3[Co(NO_2)_6]$; and for sodium with potassium pyro-antimonate, $K_2H_2Sb_2O_7$.

Carbon Monoxide

I. Detection and Estimation in Blood of Carboxyhaemoglobin in Carbon Monoxide Poisoning

Carbon monoxide, CO, is a blood poison. When inhaled, it displaces loosely bound oxygen in oxyhaemoglobin of blood and then combines with reduced haemoglobin forming carboxyhaemoglobin. Haemoglobin of blood thereby loses its power of combining with oxygen and again forming oxyhaemoglobin, the oxygen-carrier of the organism. Hüfner states that displacement of oxygen in blood by carbon monoxide takes place in accordance with the laws of chemical mass action. Hence in many poisoning cases the toxic action of carbon monoxide may be overcome by intensive artificial respiration of oxygen:

Oxyhaemoglobin + Carbon Monoxide $\rightleftarrows$ Carboxyhaemoglobin + Oxygen

Death ensues when the haemoglobin-content of the blood-pigment still free from carbon monoxide drops to 35–30 per cent., that is, when 65–70 per cent. of the oxyhaemoglobin has been converted into carboxyhaemoglobin. Consequently blood of a person poisoned by carbon monoxide can never be saturated with carbon monoxide, since death ensues before saturation takes place. It is a notable fact that carbon monoxide blood at first easily gives off carbon monoxide by passing through it a current of air or by pumping. Later it parts with the gas only with great difficulty. Affinity of oxygen for haemoglobin is said to be 210 times less than that of carbon monoxide. This explains why, in respiration of a mixture of carbon monoxide and air, blood can enrich itself with carbon monoxide even when the quantity of poison in air is very small. Gruber found that air containing 0.02 per cent. of carbon monoxide is at the limit of toxicity. When the percentage of carbon monoxide is 0.05 per cent. positive toxic action takes place. Air containing 1 per cent. of carbon monoxide rapidly exerts toxic action. The view that complete saturation of blood does not take place during life is supported by the observations of L. Smith in the case of several

persons who died from poisoning by illuminating gas and whose blood was not over 83 per cent. saturated with carbon monoxide. Illuminating gas contains 8–11 per cent. of carbon monoxide and so-called coal gas 0.3–5 per cent. Percentage of carbon monoxide in the latter varies with the nature of the coal from which it is formed.

Blood containing carboxyhaemoglobin may be recognized by its light cherry-red to violet color. The foam in particular formed by shaking has a violet color. In distinction from normal blood, putrefaction of carbon monoxide blood takes place slowly. Even after 2 months in the case of an adult, who died from breathing coal gas and whose blood was saturated with carbon monoxide up to 68 per cent., the author got a distinct test for carbon monoxide. In this instance the blood had no odor whatever of putrefaction.

A. Chemical Detection

Most of the tests recommended for detection of carbon monoxide blood depend upon difference in behavior of oxyhaemoglobin and carboxyhaemoglobin toward certain reagents. Usually oxyhaemoglobin is decolorized, whereas the reagent has no effect upon the light red color of carboxyhaemoglobin. Certain of these reagents are sodium hydroxide solution, hydrogen sulphide, potassium ferrocyanide, tannic acid, alkaline pyrogallic acid solution, and salts of heavy metals.

1. **Boiling Test.**—Blood containing carbon monoxide gives a brick-red coagulum, if boiled or warmed upon the water-bath. Ordinary blood gives a grayish brown or brownish black precipitate.

2. **Sodium Hydroxide Test.**—Carbon monoxide blood shaken with 1–2 volumes of sodium hydroxide solution (sp. gr. 1.3 = 26.8 per cent.) gives a red coagulum of carbon monoxide haematin. Normal blood similarly treated gives gelatinous oxyhaematin having a green-black to black color and in a thin coating upon a porcelain plate dark greenish brown. According to Salkowski, the best way to make this test is to dilute the blood in a test-tube with 20 times its volume of water and then add an equal volume of sodium hydroxide solution (sp. gr. 1.34). Carbon monoxide blood at first gives a milkiness and then becomes light red, whereas normal blood is changed to a dirty brown mixture.

3. **Basic Lead Acetate Test.**—Mix 4–5 volumes of basic lead acetate solution in a test-tube with diluted or undiluted carbon monoxide blood and shake well for several minutes. Such blood remains

bright red but normal blood is first brownish and then chocolate to greenish brown.

4. Potassium Ferrocyanide Test.—Mix undiluted blood (15 cc.) with an equal volume of 20 per cent. potassium ferrocyanide solution and 2 cc. of dilute acetic acid (1 vol. of glacial acetic acid + 2 vols. of water). Shake the mixture gently and a coagulum will gradually form. That from normal blood is dark brown but bright red from blood containing carbon monoxide. This difference disappears slowly but not entirely for weeks.

5. Tannic Acid Test.—Mix a solution of 1 part of blood in 4 parts of water with 3 times its volume of 1 per cent. tannic acid solution and shake well. A difference in color between normal and carbon monoxide blood may be recognized after several hours, most distinctly after 24 hours. Normal blood is gray but carbon monoxide blood is crimson-red. This difference is apparent even after several months. Ten per cent. of carboxyhæmoglobin may be detected in blood by tests 4 and 5.

6. Copper Sulphate Test.—A drop of saturated copper sulphate solution added to 2 cc. of carbon monoxide blood mixed with the same volume of water gives a brick-red precipitate. The deposit from normal blood is greenish brown. In all these precipitation tests (4, 5 and 6) the less easily decomposed carbon monoxide blood remains bright red but the more easily decomposed normal blood in presence of the precipitants used and others is off color or dark.

7. Ammonium Sulphide Test.—Mix 0.2 cc. of ammonium sulphide solution and 0.2–0.3 cc. of 30 per cent. acetic acid with 10 cc. of 2 per cent. aqueous blood solution. Carbon monoxide blood gives a fine rose color but normal blood is greenish gray. Within 24 hours the former gives a red flocculent precipitate. In the procedure of Salkowski, 20–24 drops of blood are added to 50 cc. of water and then half to three-fourths its volume of saturated hydrogen sulphide water. Carbon monoxide blood does not change color but normal blood gives a dirty green mixture owing to formation of thiohaemoglobin.

8. Palladous Chloride Test.—Carbon monoxide precipitates black metallic palladium from a neutral aqueous palladous chloride solution:

$$CO + H_2O + PdCl_2 = CO_2 + 2HCl + Pd$$

Mix a few drops of potassium hydroxide solution with the blood and warm gently upon the water-bath. By means of a suction-pump, draw through the solution air that has been washed until pure.

Pass the gas evolved first through lead acetate solution to remove possible hydrogen sulphide, then through sulphuric acid to absorb ammonia and finally through a neutral light red palladous chloride solution (1:500).

Notes.—Franzen and v. Mayer[1] have examined the various tests for detection of carbon monoxide in blood and have come to the conclusion that the best are Hoppe-Seyler's sodium hydroxide test as modified by Salkowski, and the ferrocyanide and tannic acid tests carried out according to Kunkel and Welzel. All three tests make it possible to detect 1 per cent. of carbon monoxide in blood with certainty. The color change in the tannic acid test appears only after several hours, whereas the sodium hydroxide and potassium ferrocyanide tests may be seen very clearly even after a short time. For this reason the two latter tests deserve first consideration in the practical detection of small quantities of carbon monoxide.

B. Spectroscopic Detection

Detection of carboxyhaemoglobin by the spectroscope is comparatively easy. The two absorption-bands of this compound are quite similar to those of oxyhaemoglobin but lie somewhat nearer together and more toward the violet. The main difference, however, between the absorption-bands of these compounds is that those of carboxyhaemoglobin are not extinguished by reducing agents. To prepare the blood solution for spectroscopic examination, dilute 1–1.5 parts of blood with 100 parts of water and make the observations through a layer 1 cm. thick. To reduce 1 per cent. blood solution, mix well with 1–2 cc. of ammonium sulphide solution and add 4–6 drops more of the same reagent as a surface-layer to exclude air. In the case of normal blood, reduction begins in about 2–3 minutes. A solution of tartaric acid and ferrous sulphate in presence of excess of ammonium hydroxide solution will also reduce oxyhaemoglobin.

Under these conditions oxyhaemoglobin is changed to reduced haemoglobin. The two absorption-bands characteristic of the former disappear and a broad diffuse absorption-band occupies the previous bright space between the two bands. The spectrum of carboxyhaemoglobin remains unchanged only when 26.5 per cent. at least of the haemoglobin is saturated with carbon monoxide. If allowed to stand in an open vessel, blood will lose carbon monoxide within 8 days. But carbon monoxide blood sealed in glass tubes is said to keep for years. Carbon monoxide has been detected spectroscopially in blood of a cadaver after 18 months.

[1] H. Franzen and O. v. Mayer: Detection of Carbon Monoxide with Blood. Zeitschr. f. analyt. Chem. 50 (1911), 669.

Tollens[1] recommends adding formaldehyde to the blood solution to be examined spectroscopically. This reagent has not the slightest effect upon the two oxyhaemoglobin bands. Warming the mixture very gently with ammonium sulphide solution develops a third and nearly as distinct black band almost midway between the original bands which gradually disappear. Finally only this band will remain. This is a far more satisfactory test than that given by the indefinite band of blood alone. If the solution is cooled and agitated with air, this third band will disappear and the two original oxyhaemoglobin bands return.

In presence of carboxyhaemoglobin, formaldehyde does not have this action. Then upon warming with ammonium sulphide the two bands of carboxyhaemoglobin displaced somewhat toward the oxyhaemoglobin bands remain even after

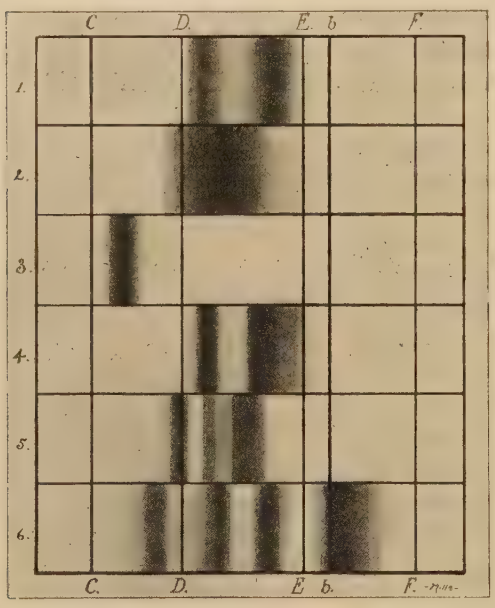


FIG. 27.—Absorption-spectra.

addition of formaldehyde. Mix 15–30 drops of blood with 100 cc. of water, adding always a few drops of yellow ammonium sulphide. Then add 3–4 drops of approximately 40 per cent. formaldehyde solution either before or after ammonium sulphide.

C. Approximately Quantitative Estimation of Carboxyhaemoglobin in Carbon Monoxide Blood

Frequently the forensic chemist is called upon to decide whether death due to poisoning from inhalation of illuminating gas, or coal

[1] R. Tollens: Spectroscopic Test for Blood in Presence of Formaldehyde. Ber. d. Deutsch. chem. Ges. 34 (1901), 1426.

gas, should be ascribed exclusively to poisoning from carbon monoxide. The answer to this question depends upon the quantitative estimation of carboxyhaemoglobin, since according to statements made above blood must be saturated with carbon monoxide to a definite degree before death from asphyxiation occurs. In an investigation of this character, the author adopts Welzel's simple method which gives a result quickly and is of sufficient accuracy for practical purposes. Experiments with blood containing a known quantity of carbon monoxide have shown that blood containing not over 26.5 per cent. of carbon monoxide behaves spectroscopically toward reducing agents exactly like normal blood, that is, shows a broad diffuse band. Consequently, if enough normal blood is added to the blood in which carboxyhaemoglobin is to be determined just to produce the result mentioned, the quantity of carboxyhaemoglobin may be calculated from the quantity of normal blood required. Experiments with this method showed that rabbits die when their blood is three-quarters saturated with carbon monoxide.

II. Detection and Estimation of Carbon Monoxide in Air

Of the many methods recommended for detection of carbon monoxide in air, the two most likely to give a reliable result are: I. That depending upon formation of carboxyhaemoglobin and its spectroscopic recognition; II. That based upon the reduction of palladous chloride solution by carbon monoxide.

Method I.—Shake one or several liters of air with about 5 cc. of an aqueous solution of normal blood, diluted so that it still appears to have a faint red color and yet permits the normal blood spectrum to be distinctly recognized. In presence of carbon monoxide, the blood solution changes color and at the same time behaves differently when examined spectroscopically after addition of ammonium sulphide. This test may be rendered more delicate by drawing 10–20 liters of suspected air by an aspirator through a few cc. of diluted blood solution and then examining it spectroscopically. These directions given by Hempel permit detection of even 0.05 per cent. of carbon monoxide in air. By passing the gas through two glass funnels held together by a rubber ring and placing a live mouse in the enclosure thus formed, Hempel has rendered this method exceedingly delicate. The gas is passed through for 3–4 hours at the rate of 10 liters per hour. The mouse is killed by dipping the funnels

under water. A few drops of blood are taken from the region of the heart, diluted with water and tested spectroscopically. In this manner Hempel has detected with certainty even 0.032 per cent. of carbon monoxide. No toxic symptoms from these small quantities of carbon monoxide are visible. They do not appear until the quantity of carbon monoxide is 0.06 per cent. and then after about half an hour. The mouse has dyspnoea and lies on its side exhausted. This test for carbon monoxide may be negative in presence of acid vapors and even of considerable quantities of carbon dioxide. For this reason the gas before entering the funnels should be passed through 2–3 wash-bottles filled with potassium hydroxide solution.

Method II.—Pass about 20 liters of air through 50 cc. of diluted blood. Immediately heat this blood solution in a flask at 90–95° and draw slowly through for 3–4 hours a current of air washed through dilute palladous chloride solution. Oxygen of the air by mass action gradually displaces carbon monoxide from its union with haemoglobin. The current of air containing carbon monoxide after leaving the blood solution is purified by being drawn first through dilute sulphuric acid, then through lead acetate solution, and finally through neutral, dilute palladous chloride solution having a light, yellow-red color. If air contains carbon monoxide, a black precipitate of metallic palladium appears. Gaglio states that even rather old blood will give useful results, if pieces of potassium hydroxide, or its solution, are added. The advantage of this method is that blood then does not coagulate. A more rapid method of detecting carbon monoxide in air is to use palladous chloride paper. This is moistened and suspended in a flask containing some water and filled with the given air. The flask for this purpose should have a volume of at least 10 liters. Even after a few minutes 0.05 per cent. of carbon monoxide gives a black, shiny film upon the paper; 0.01 per cent. after about 4 hours; and 0.001 per cent. after 20–24 hours. Sensitive as this test is, it is not characteristic, since hydrogen sulphide, ammonia, other components of illuminating gas, and also sunlight cause darkening of palladous chloride paper. Consequently this test is significant only when negative.

Gadamer's[1] Quantitative Estimation of Carbon Monoxide

To obviate the disadvantages mentioned, Gadamer first absorbs carbon monoxide in hydrochloric acid solution of cuprous chloride

[1] J. Gadamer: Lehrbuch der chemischen Toxikologie. 1909, page 46.

and then allows this product to react with palladous chloride solution. The precipitate of metallic palladium is finally weighed.

Procedure.—Wash a measured volume of air with lead acetate solution and then pass through hydrochloric acid solution of cuprous chloride containing finely divided metallic copper. Filter into palladous chloride solution, disregarding any precipitate that appears, and add ammonia in excess. Metallic palladium separates almost at once and copper salts pass into solution. Excess of palladous chloride is of course required. Collect the palladium precipitate in a weighed asbestos filter-tube, or Gooch crucible, wash with ammonia, and ignite after having dried first in a stream of hydrogen and then in air. Finally weigh crucible and palladium.

Calculation.—According to the equation: $PdCl_2 + H_2O + CO = Pd + 2HCl + CO_2$, 1 mg. of palladium corresponds to 0.263 mg. of carbon monoxide calculated from Pd (106.5):CO (28) = 1:x (x = 0.263).

Under normal conditions a molecular volume of carbon monoxide occupies a volume of 22400 cc. According to the equation: 28:22400 = 0.000263:x (x = 0.2104), 1 mg. of palladium corresponds to 0.2104 cc. of carbon monoxide at 0° and 760 mm.

According to Gadamer, this method of estimation is not entirely free from objection, since occasionally, for reasons as yet unexplained, even cuprous chloride solution alone causes precipitation of palladium. To avoid error, Gadamer recommends always making a blank test with air free from carbon monoxide under precisely the same conditions that obtain in the actual test.

B. ORGANIC SUBSTANCES

Organic Arsenical Compounds

In general the toxic action of arsenic in inorganic combination, known also as "mineral arsenic," upon man and higher animals is considerably greater than that of the same quantity of arsenic in organic combination. The minimal lethal dose of As_2O_3, alkali arsenites and sodium arsenate (Na_2HAsO_4) contains 0.0037 gram of arsenic per kilogram of rabbit, whereas for atoxyl the quantity is 0.072 gram. The dose, causing no actual acceleration of death upon further increase, contains 0.018 gram of arsenic for the inorganic arsenic compounds mentioned per kilogram of rabbit, whereas for atoxyl the quantity is 0.2 gram. Certain organic arsenical compounds, because of lower toxicity for man, have for a number of years found use in medicine, especially in those cases where prolonged action from arsenic administered by the mouth, subcutaneously, or intravenously is desired. The substances to be considered

are derivatives of quinquevalent arsenic, that is, of arsenic acid (H_3AsO_4) through substitution of organic radicals for one or two hydroxyl groups. The most important compounds of this series because of use in medicine are:

1. **Arrhenal,** or Sodium Methyl Arsinate, $CH_3.AsO(ONa)_2.6H_2O$.
2. **Sodium Cacodylate,** or Sodium Dimethyl Arsinate, $(CH_3)_2AsO(ONa).2H_2O$.
3. **Atoxyl** (Arsanilate), or Sodium Para-arsanilate, $H_2N.C_6H_4.AsO(ONa)(OH).4H_2O$.
4. **Arsacetine,** or Sodium Acetyl-para-arsanilate, $CH_3.CO.NH.C_6H_4.AsO(ONa)(OH).4H_2O$.
5. **Salvarsan,** Ehrlich-Hata 606, or 3,3′-Diamino-4,4′-dihydroxy-arsenobenzene Dihydrochloride, $HCl.NH_2.C_6H_3(OH).As = As.C_6H_3(OH).NH_2HCl$.

Organic compounds of arsenic most commonly used in medicine subcutaneously, or intravenously, such as atoxyl and cacodylic acid, are for the most part eliminated unchanged rather quickly by way of the urine. For complete elimination, however, usually several months are required. In the case of several persons who had taken the salvarsan treatment and died 2–5 months after the last injection, the author found in the liver determinable quantities of arsenic. In view of the extensive use of salvarsan by luetics, the forensic chemist should proceed with the greatest caution in expressing an opinion concerning a positive result for arsenic. Having found arsenic in any part whatsoever of the cadaver, he should not decide that it is due to arsenical poisoning until he has acquainted himself with every detail of the case. It especially becomes his duty to ascertain whether arsenic found may not have been acquired from therapeutic use of salvarsan, atoxyl, or some other organic compound of arsenic. Such medical use, however, may not have been of recent date.

Cacodylic Acid

Bunsen[1] prepared cacodylic acid, $(CH_3)_2AsO.OH$, by oxidizing cacodyl, or better cacodylic oxide, under water with mercuric oxide:

$$\begin{matrix}(CH_3)_2As\\(CH_3)_2As\end{matrix}\!\!>\!O + H_2O + 2HgO = 2Hg + 2(CH_3)_2As\!\!<\!\!\begin{matrix}O\\OH\end{matrix}$$

The solution filtered from mercury is evaporated to dryness with moderate heat and the residue recrystallized from hot alcohol.

Cacodylic acid forms colorless, inodorous, rhombic crystals, melting at 200° and readily soluble in water. The resistance it

[1] R. Bunsen: Researches upon Cacodyl Compounds. Annal. d. Chemie und Pharm. 46 (1843), 2.

offers to oxidation is extraordinary. Fuming nitric acid, aqua regia, chromic acid and potassium permanganate are without action. Potassium chlorate together with hydrochloric acid has no effect upon it. Phosphorous acid reduces it to cacodylic oxide. Cacodylic acid is monobasic, its salts for the most part being readily soluble in water. Sodium cacodylate, $(CH_3)_2AsO.ONa$, is a white crystalline powder freely soluble in water. Mercuric cacodylate, $[(CH_3)_2AsO.O]_2Hg$, obtained by dissolving freshly precipitated yellow mercuric oxide in aqueous cacodylic acid and allowing the solution to evaporate spontaneously, forms fine, white, crystalline needles that easily decompose. Cacodylic acid and its salts are characterized by low toxicity. They are slowly eliminated unchanged in greatest part by way of the urine. Cacodylic acid with nascent hydrogen, especially with zinc and sulphuric acid, gives white fumes having the odor of cacodylic oxide and coloring silver nitrate solution red-brown. Arsine is not set free. This is not evolved unless cacodylic acid has previously been boiled for several hours with concentrated sulphuric acid. Cacodylic acid heated with magnesium powder gives off a strong odor of cacodyl.

Arsenic in inorganic combination is readily distinguished from cacodyl arsenic by the property possessed by cacodylic acid of not forming yellow arsenic sulphide with hydrogen sulphide even after treatment with potassium chlorate and hydrochloric acid, or other oxidizing agents. On the other hand, when examined in the Marsh apparatus, cacodylic acid gives a yellow-red ring characteristic of this arsenic compound. In the reduction tube, it also evolves dimethylarsine, $(CH_3)_2AsH$, still recognizable by its cacodyl odor even when only 0.01 mg. is present (Ganassini[1]). Cacodylic acid, although extraordinarily stable toward oxidizing agents, when introduced in small quantities into fused potassium nitrate is converted for the most part into potassium arsenate.

Baumert's Modification of Vitali's[2] Procedure for Detection of Cacodylic Acid in Urine and Parts of the Cadaver

Acidify the material (urine and organs) with tartaric acid, evaporate to dryness upon the water-bath, and extract the residue with

[1] D. Ganassini: Cacodylic Acid and Its Toxicological Detection. Boll. Chimic. Farmacolog. 42 (1903), 5.

[2] D. Vitali: Elimination of Cacodylic Acid and Its Detection in Poisoning Cases. Boll. Chim. Farmacol. 40 (1901), 657.

90 per cent. alcohol. Filter this extract and distil off the alcohol. Add chloroform to the residue and sufficient alcohol to bring all the chloroform into solution. Addition of water to this alcohol-chloroform mixture precipitates cacodylic acid held in solution by chloroform. Separate this chloroform solution, distil off the chloroform, and test the residue for cacodylic acid. It appears as prismatic crystals readily soluble in water, methyl or ethyl alcohol, and chloroform. Aqueous solutions give the penetrating cacodyl odor with zinc and dilute sulphuric acid, phosphorous acid, hypophosphorous acid, and also with Bougault's reagent. The same odor is developed by fusion with potassium persulphate, or by heating with magnesium powder. Finally the solution of the residue may be examined in the Marsh apparatus.

Methyl Arsinic Acid and Arrhenal

Cacodylic acid is the best substance to use for the preparation of methyl arsinic acid. Treated with phosphorus pentachloride, it is converted into cacodyl-trichloride through replacement of O and OH by chlorine. Warmed to 40–50°, the latter loses methylchloride and is converted into methylarsine dichloride. This compound reduced by means of amalgamated zinc dust and alcoholic hydrochloric acid gives off methylarsine, an exceedingly poisonous gas. Methyl arsinic acid is formed from this gas by oxidation and passing it into neutral silver nitrate solution:

$$(CH_3)_2AsO.OH + PCl_5 \rightarrow (CH_3)_2AsCl_3 \xrightarrow{\text{At } 40^\circ \text{ to } 50^\circ} CH_3.AsCl_2$$

Cacodylic acid — Cacodyl-trichloride — Methylarsine dichloride

$$Zn + HCl \rightarrow CH_3.AsH_2 \xrightarrow{\text{Oxidation}} CH_3.As(=O)(OH)_2$$

Methylarsine — Methyl arsinic acid

Methyl arsinic acid crystallizes from alcohol in colorless leaflets melting at 161°.

Arrhenal.—This compound is the disodium salt of methyl arsinic acid, $CH_3.AsO(ONa)_2.6H_2O$, obtained by dissolving As_2O_3 and sodium hydroxide in a mixture of water and alcohol, adding methyl iodide, and allowing the clear mixture to stand in a closed vessel for several days:

$$CH_3I + NaAsO(ONa)_2 = NaI + CH_3.AsO(ONa)_2$$

Arrhenal forms colorless crystals, dissolving readily in water but with difficulty in alcohol. These crystals effloresce in air and so vary in the quantity of water they contain. An aqueous solution is alkaline and is precipitated by silver nitrate, copper sulphate, lead acetate and mercuric chloride. These precipita-

tion reactions serve to distinguish arrhenal from sodium cacodylate. Among other differences the following may be mentioned. In the Marsh apparatus arrhenal, in absence of platinic chloride, gives rise to black rings not preceded by appearance in the evolution flask of white vapor having a garlic odor, as is the case with cacodylic acid. Warmed with Bettendorff's reagent, cacodylic acid gives a white vapor and garlic odor but no coloration. Arrhenal gives no reaction, or only a yellow coloration. But on longer heating a white sublimate, later turning yellow, and finally becoming brown, forms upon the upper part of the tube.

Ten times the quantity of hypophosphorous acid (sp. gr. 1.15) with cacodylic acid at once produces an intense cacodyl odor, whereas arrhenal remains odorless.

Quantitative Estimation of Arsenic in Cacodylic Acid and Arrhenal

The difficulty attending the breaking up of the combination in case of these two organic arsenic compounds and reconverting arsenic into an inorganic compound is very great. The method recommended by d'Emilio[1] certainly should bring about this change. Boil for about 1 hour over a free flame in a Kjeldahl flask 0.2–0.3 gram of substance with 10 cc. of arsenic-free, concentrated sulphuric acid and 10 grams of potassium sulphate, adding occasionally a little powdered potassium permanganate. Cool, dilute with water, neutralize with ammonia to faint acid reaction, and precipitate arsenic with hydrogen sulphide. Since the precipitate always consists of a mixture of As_2S_3 and As_2S_5 and free sulphur, it is advisable to dissolve the filtered and washed precipitate in ammonia upon the paper. Evaporate the solution in a porcelain dish and repeatedly evaporate the residue with strong bromine water to effect complete conversion of arsenious to arsenic acid. Finally precipitate arsenic acid thus obtained with magnesia mixture and weigh as magnesium pyroarsenate, $Mg_2As_2O_7$.

To estimate cacodylic acid in urine quantitatively, Heffter[2] evaporates the latter rendered alkaline with sodium hydroxide solution and gradually introduces the residue, mixed with 1 part of potassium hydroxide and 5 parts of potassium nitrate, into a glowing crucible. Dissolve the cold melt in water and excess of hydrochloric acid and boil for some time, to remove nitrous acid completely and most of the nitric acid. Then precipitate arsenic with hydrogen sulphide, convert the precipitate as directed above into arsenic acid,

[1] L. d'Emilio: Organic Arsenic in Therapy. Cacodylic Acid, Methyl Arsinic Acid and Its Salts. Boll. Chim. Farm. 41 (1902), 633.

[2] A. Heffter: Behavior of Cacodylic Acid in the Organism and Detection in Urine. Schweiz. Wochenschr. Pharm. 39 (1901), 193.

and finally weigh magnesium pyroarsenate, $Mg_2As_2O_7$. In the opinion of many authors, large quantities of cacodylic acid escape oxidation by the fusion mixture of potassium hydroxide and potassium nitrate and for this reason Heffter's method gives too low results.

Notes.—After the last dose of cacodylic acid administered by the mouth, elimination by way of the urine continues for a long time. In fact it has been observed to be taking place after 70 days. The cacodyl odor of the urine is about the best means of recognizing presence of cacodylic acid (Barthe and Péry[1]).

Atoxyl

Atoxyl is the sodium salt of para-arsanilic acid, $H_2N.C_6H_4$-$AsO(OH)(ONa).4H_2O$, formed by heating a mixture of aniline and arsenic acid, consequently aniline arsenate, up to 180–200° with elimination of water:

$$H_2N.C_6H_4H + HOAsO(OH)_2 \rightarrow H_2O + H_2N.C_6H_4AsO(OH)_2$$

Dissolve the product of this reaction in sodium carbonate solution, filter and saturate with hydrochloric acid. Free para-arsanilic acid is precipitated and recrystallized from hot water. The shining white needles thus obtained are soluble with difficulty in cold water and in alcohol, easily soluble in boiling water and in methyl alcohol, and almost insoluble in ether, chloroform and acetone. Neutralization of free acid with one equivalent of sodium carbonate or bicarbonate gives atoxyl. The free acid, however, also has weak basic properties, for with hydrochloric acid it forms the salt $HCl.H_2N.C_6H_4$-$AsO(OH)_2$.

Atoxyl is a white, crystalline, inodorous powder, soluble in about 6 parts of water at 17° and very freely soluble in hot water with neutral reaction. Almost insoluble in alcohol, it is easily soluble when anhydrous in methyl alcohol. Presence of a free amino-group in atoxyl explains the solubility of free para-arsanilic acid in dilute mineral acids, the easy formation of an acetyl derivative, and conversion by means of hydriodic acid into para-iod-aniline.

Therapeutic Use.—As compared with inorganic arsenic compounds, atoxyl is characterized by low toxicity. It is a specific for sleeping sickness and to some extent also for lues. It is further recommended in treatment of skin diseases, anaemia and tuberculosis.

[1] L. Barthe and R. Péry: Elimination and Toxicological Detection of Cacodylic Acid. J. Pharm. Chim. (6) 13 (1901), 209.

Detection of Atoxyl[1]

A. **Arsenic.**—Carefully heated in a fusion tube, atoxyl gives a garlic-like odor and an arsenic mirror. Hydrogen sulphide passed for a long time into a hot solution of atoxyl precipitates first sulphur and then yellow arsenic sulphide. In the Marsh and Gutzeit tests it behaves like inorganic arsenic compounds. The Reinsch test also gives a distinct result though rather slowly. Consequently atoxyl cannot be present when these three tests are negative! On warming, Bettendorff's and Bougault's reagent gradually give lemon-yellow but not brownish precipitates. Distilled according to Schneider-Fyfe-Beckurts in a stream of hydrochloric acid, atoxyl does not give arsenic trichloride. It will yield the latter, however, when distillation takes place in presence of ferrous chloride.

B. **The Primary Aromatic Amino-Group.**—As a primary aromatic amine, atoxyl can be diazotized and made to form azo-dyes by coupling its diazonium salt with phenols, such as resorcinol and β-naphthol, and also with α- and β-naphthylamine. For example, diazotized p-arsanilic acid gives with β-naphthylamine a red, crystalline precipitate of the azo-dye $HCl.NH_2.C_{10}H_6N = N.C_6H_4.AsO(OH)_2$,[2] readily soluble with red color in cold sodium carbonate solution and reprecipitated from this solution by acids.

Procedure.—Add a few drops of sodium nitrite solution (1:10) to an aqueous atoxyl solution, cool, acidify with dilute hydrochloric or sulphuric acid, and add to this diazonium solution a few drops of a hydrochloric acid solution of α- or β-naphthylamine. The α-derivative gives a purple-red color and the β-derivative more of a brick-red color that soon forms a colored precipitate leaving the solution nearly colorless. Atoxyl may also be precipitated quantitatively by means of the latter reaction. Neither reaction, however, in pure aqueous solutions is characteristic, since the naphthylamines themselves even in absence of atoxyl give purple-red colorations due to diazotization of a part of the base and self-coupling. Consequently after diazotization of aqueous atoxyl solutions, or aqueous extracts of organs, sufficient urea must be added to decompose all

[1] J. Gadamer: Atoxyl in the Forensic Detection of Arsenic. Apotheker-Zeitung 22 (1907), 566.

[2] P. Ehrlich and A. Bertheim: Para-arsanilic Acid. Ber. d. Deutsch. chem. Ges. 40 (1907), 3292.

free uncombined nitrous acid, as shown by failure to produce a blue color on starch iodide paper:

$$CO(NH_2)_2 + 2HONO = CO_2 + 2N_2 + 3H_2O$$
$$2HONO + 2HI = 2NO + 2H_2O + I_2$$

Then add the naphthylamine solution. This reaction may be applied to urine direct, for it contains enough urea to take care of any excess of nitrous acid in the diazonium solution. An alkaline resorcinol or β-naphthol solution, added to diazotized atoxyl solution so that the reaction is alkaline in the end, produces a purple-red color due to formation of an azo-dye. Although these tests are somewhat less sensitive, they are more reliable and more characteristic than those given by the naphthylamines.

The reactions under (B) merely show presence of a primary aromatic amino-compound. They are conclusive for presence of atoxyl or salvarsan only when the azo-dye is precipitated, washed and shown to contain arsenic. To make this test, filter the brick-red precipitate obtained by means of the solution of β-naphthylamine hydrochloride, wash, dry, and by fusion with sodium nitrate, or magnesium oxide (see Chapter V, page 493), convert arsenic into arsenic acid, testing the product in the Marsh apparatus. If sodium nitrate was used for the fusion, first completely expel nitric and nitrous acids from the solution of the melt by heating with arsenic-free sulphuric acid until copious white fumes are given off. Gadamer detects atoxyl in urine and parts of the cadaver in the following manner. Having decolorized strongly colored urine with a little freshly ignited animal charcoal, acidify with sulphuric acid, cool, and test for formation of azo-dye by reactions given above under (B). Atoxyl is not present, unless dyestuff is formed. If the test is positive, frequently a further test must be made to determine whether mineral arsenic (arsenious or arsenic acid) is not present too. To do this, completely precipitate atoxyl with β-naphthylamine as under (B), and test the filtrate for arsenic in the usual manner after having first destroyed organic matter with hydrochloric acid and potassium chlorate.

Comminute organs, or other parts of the cadaver, add to the material many times its volume of alcohol, render just acid with sulphuric acid, and warm for several hours. Filter, evaporate the solution, gradually but completely precipitate the syrupy residue with absolute alcohol, and expel alcohol from the filtered solution by distillation or evaporation. Dissolve the residue in water and examine the filtered solution as follows:

1. First test for arsenic by means of the Reinsch, Marsh or Gutzeit test. If the test is negative, atoxyl is absent.

2. If test (1) is positive, add 3 vols. of Bettendorff's reagent to 1 vol. of the solution and warm gently. If this reagent gives no reaction, or only separation of a lemon-yellow precipitate, atoxyl may be present but no mineral arsenic. To test with greater certainty for atoxyl, try the diazo-reactions given under (B). In tests with naphthylamines excess of nitrous acid should be removed by means of urea before naphthylamine hydrochloride solution is added. Arsacetine, or

acetyl-atoxyl, does not give the diazo-reaction until after treatment with strong hydrochloric acid or sodium hydroxide solution.

If Bettendorff's reagent produces a brown precipitate with the original solution, mineral arsenic is present.

Elimination of Atoxyl in Urine.—Croner and Seligmann[1] found that atoxyl after subcutaneous injection was eliminated very quickly mainly through the urine, that is, within 24 hours, and almost wholly unchanged. For days traces of atoxyl may be detected in urine. Elimination of atoxyl is prolonged after injections repeated at short intervals. The azotiring method of Ehrlich and Buthein may be used to detect and estimate atoxyl in urine. With β-naphthylamine the diazonium compound of atoxyl gives a carmine-red precipitate having the composition, $HCl.NH_2C_{10}H_6.N = N.C_6H_4.AsO(OH)_2$. Addition of sodium acetate solution forms, instead of the hydrochloride, the free amino-compound having a more purple-red color.[2] The limit of delicacy of atoxyl detection by precipitation as azo-dye is 1 mg. in 100 cc. of water, or a dilution of 1:100,000. In case of urine this proportion does not always hold, since presence of other substances interferes. Elimination of atoxyl after subcutaneous injection in man is nearly at an end after 9 hours. Within this period in individual cases atoxyl as such leaves the organism almost quantitatively. Usually eliminaton lies between 50–90 per cent. Part of the atoxyl taken undergoes a change within the human body. Elimination of arsenic present in urine not as atoxyl may be of several days duration (Igersheimer and Rothmann[3]). These authors found that atoxyl dissolved in the serum circulates through the body but that apparently the corpuscles have only a slight affinity for the poison. Appreciable quantities of a reduction product of atoxyl are not present in urine but most of this compound is eliminated as such.

Acetyl-Para-Arsanilic Acid and Arsacetine[4]

A mixture of sodium p-arsanilate (=atoxyl) and acetic anhydride becomes warm when stirred and actively boils. Momentarily everything passes into solution and then solidifies to a mass of crystals. Stir with dilute hydrochloric acid when cold, suck off the mother-liquor after several hours, and wash with water, alcohol and ether. Acetyl-arsanilic acid, or acetyl-p-amino-phenyl-arsinic acid, $CH_3.CO.NH.C_6H_4.AsO(OH)_2$, thus obtained forms shining, white leaflets, has scarcely any basic properties, and so is precipitated unchanged by water from solution in concentrated hydrochloric acid. It may also be prepared by boiling atoxyl for several hours with glacial acetic acid. By introducing acetyl-arsanilic acid into warm concentrated sodium hydroxide solution to the point of neutralization, its sodium salt, arsacetine, $CH_3.CO.NH.C_6H_4.AsO(ONa)(OH)5H_2O$, crystallizes as the solution cools in fine, white needles easily soluble in water and in methyl alcohol. Precipitation of the aqueous solution with alcohol gives an arsacetine crystalliz-

[1] Croner and Seligmann: Deutsche med. Wochenschrift 33,995.

[2] G. Lockemann and M. Paneke: Detection and Process of Elimination of Atoxyl in Urine. Deutsche med. Wochenschrift 34 (1908), No. 34.

[3] J. Igersheimer and A. Rothmann: Behavior of Atoxyl in the Organism. Quantitative Estimation of Atoxyl. Zeitschr. f. physiol. Chemie 59 (1909), 256.

[4] P. Ehrlich and A. Bertheim: Para-arsanilic Acid. Ber. d. Deutsch. chem. Ges. 40 (1907), 3292.

ing with $4H_2O$. Boiling caustic alkalies hydrolyze it, forming arsanilic acid and acetic acid:

$$CH_3.CO.NH.C_6H_4.AsO(ONa)(OH) + KOH = CH_3.COOK + H_2N.C_6H_4.AsO(ONa)(OH)$$

Introduction of the acetyl-group greatly diminishes the toxicity of atoxyl, for arsacetine is considerably less toxic than atoxyl. At the same time Ehrlich found it to be equal to the latter in curative powers for many animal species. Solutions of arsacetine sterilized in autoclave at 130° undergo no decomposition.

Estimation of Arsenic in Atoxyl and Arsacetine

(Rupp and Lehmann[1])

Warm 0.2 gram of material at 70° in a 200 cc. flask with 10 cc. of concentrated sulphuric acid. Then while shaking the contents, add in small portions at a time 1 gram of crystallized potassium permanganate. The mixture is decolorized by dropping in 5–10 cc. of officinal 3 per cent. hydrogen peroxide. Dilute with 20 cc. of water, boil for 15 minutes, and add 50 cc. more of water. Cool and add 2 grams of potassium iodide. Allow to stand for 1 hour and titrate free iodine without use of indicator.

Salvarsan

Salvarsan, or Ehrlich-Hata 606, is 3.3′-diamino-4.4′-dihydroxy-arseno-benzene dihydrochloride, having the composition:

$$HCl.NH_2.C_6H_3(OH).As = As.C_6H_3(OH).NH_2.HCl + 2H_2O$$

Preparation.—Phenol heated with sulphuric acid is converted into phenol-p-sulphonic acid and similarly treated with arsenic acid is changed to phenol-p-arsinic acid:

$$HO.C_6H_4.H + HO.AsO(OH)_2 = H_2O + HO.C_6H_4.AsO(OH)_2 (1, 4)$$

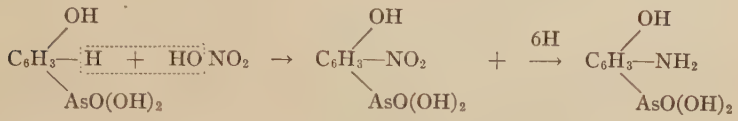
By the combined action of nitric and sulphuric acid at 0° this arsinic acid is nitrated to nitro-phenol-arsinic acid which like all nitro-derivatives is reduced by nascent hydrogen, best by sodium hydrosulphite[2] in alkaline solution, or by metal-

[1] E. Rupp and F. Lehmann: Simplified Estimation of Arsenic in Atoxyl and Arsacetine. Apotheker-Zeitung 26 (1911), 203.

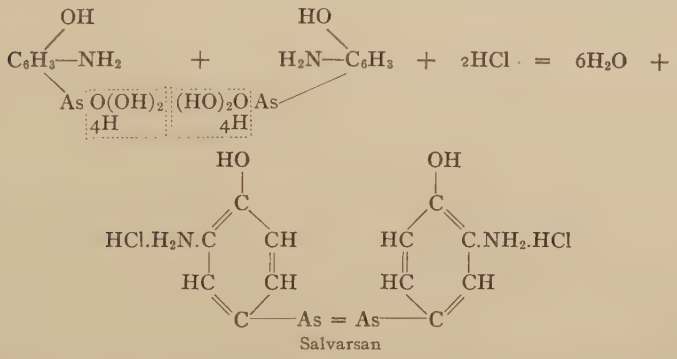
[2] Sodium hydrosulphite is the sodium salt of hydrosulphurous or dithionous acid. The sodium salt is formed by treating a concentrated solution of sodium acid sulphite with zinc filings. In the formation of the free acid, or of the sodium salt, no hydrogen is evolved. It is a very unstable compound, a strong reducing agent, rapidly absorbs oxygen from the air, becoming sulphurous acid or a sulphite. According to Bernthsen (Ber. d. Deutsch. chem. Ges. 14 (1881), 438), the sodium salt does not contain hydrogen. He gives the formula as $Na_2S_2O_4$:

$$Zn + 4NaHSO_3 = ZnSO_3 + Na_2SO_3 + Na_2S_2O_4 + 2H_2O.$$

lic sodium in methyl alcohol solution, to amino-phenol-arsinic acid difficultly soluble in water:



Further reduction of amino-phenol-p-arsinic acid with sodium hydrosulphite, or more energetic reduction of the original nitrated acid with a larger quantity of hydrosulphite, gives diamino-dioxy-arseno-benzene as a light yellow, crystalline, easily changeable powder. As a di-primary base this compound is soluble in dilute acids and as a diacid phenol is soluble in aqueous solutions of the caustic alkalies. Salvarsan is the dihydrochloride of diamino-dioxy-arseno-benzene.



Salvarsan

Salvarsan is a light yellow, crystalline powder slowly dissolving in water with acid reaction. It is easily soluble in methyl alcohol and in glycerol, somewhat more difficultly soluble in alcohol (1:12), and insoluble in ether. It contains 31.6 per cent. of arsenic. Hydrogen sulphide fails to precipitate arsenic sulphide from salvarsan solutions acidified with hydrochloric acid. Bettendorff's reagent gives merely a yellow, amorphous precipitate but not brown-black arsenic. Warmed with hydrochloric acid and potassium chlorate, until the solution is water-clear, salvarsan then gives the arsenic reactions with these two reagents. By the Marsh, Gutzeit, Schneider and Fyfe, Reinsch, and also biological test, it is possible to show presence of arsenic in salvarsan. In salvarsan solutions ferric chloride produces a coloration of green to red even in dilution of 1:15,000. Gold chloride solution gives at once an intense red coloration. Iodine solution oxidizes salvarsan to amino-phenol-arsinic acid:

$$(HO)C_6H_3(NH_2).As = As.(NH_2).C_6H_3(OH) + 6H_2O + 8I =$$

$$2(HO)C_6H_3(NH_2).As\begin{matrix}OH\\ \diagup\\ O\\ \diagdown\\ OH\end{matrix} + 8HI$$

Since 8 atoms of iodine to 1 molecule of salvarsan enter into this reaction, it may be employed to determine it volumetrically. As an aromatic, diprimary amine, salvarsan gives the diazo reaction. Unlike atoxyl, however, it fails to give this test with β-naphthylamine though it does with α-naphthylamine. To make the diazo test, acidify the salvarsan solution, with a few drops of hydrochloric acid, cool to 0°, add a slight excess of 0.5 per cent. sodium nitrite solution, removing nitrous acid by adding urea in small portions at a time until a drop of the solution no longer gives a blue color with starch-iodide paper. Finally add a saturated aqueous solution of α-naphthylamine acidified with hydrochloric acid. The beautiful ruby to violet-red coloration that appears increases in intensity on longer standing. Formation of this dyestuff is considerably hastened by heat. Salvarsan in dilution of 1:15,000 gives the red coloration in the cold after several hours. This diazo-reaction needless to say is conclusive for salvarsan only when presence of arsenic in the original solution, or better in the dyestuff formed, is established at the same time. To do this, saturate the red solution with salt as soon as gentle warming causes no further increase in intensity of color. The dyestuff is precipitated and after several hours may be filtered off, washed with saturated salt solution, dissolved in hot dilute hydrochloric acid, and tested by the Reinsch or Gutzeit test for arsenic.

Fate of Salvarsan in the Human Organism.—Shortly after administration, salvarsan is eliminated through the kidneys in the urine. But elimination of arsenic (!) does not cease for weeks. In the latter stage considerable quantities of arsenic always remain longer in the liver, and smaller quantities in the kidneys and spleen. Following intramuscular injection, arsenic is also stored in the injected muscle. In salvarsan poisonings terminating fatally the author has failed to find any arsenic in the brain, or at most traces. Examination of parts of the body for arsenic followed the usual forensic methods, organic matter being most effectively destroyed by means of hydrochloric acid and potassium chlorate. Diazotized salvarsan may of course be coupled to form azo-dyestuffs not only with α-naphthylamine but with phenols, such as resorcinol, phloroglucinol, α- and β-naphthol, as well as orcinol[1]. Resorcinol and orcinol give the most beautiful dyestuffs. An aqueous solution of salvarsan treated with Ehrlich's

[1] Orcinol is 1-Methyl-3, 5-dioxy-benzene.

diazo-reagent, that is, a mixture of a hydrochloric acid solution of sulphanilic acid and sodium nitrite solution, and a few drops of ammonia gives a brown-red coloration. Caution is necessary, since the urine of patients treated with salvarsan gives with the Ehrlich diazo-reaction a color that is only slightly different in shade. The other substances in urine do not interfere with the resorcinol test of diazotized salvarsan. After diazotization atoxyl gives an orange color with resorcinol.

In view of the extensive therapeutic use of salvarsan, the forensic chemist should bear in mind that a considerable quantity of arsenic may be found in a cadaver. He should also take into account the fact that the human body may retain arsenic for quite a long time after its use medicinally in the form of an organic compound. Müller[1] cites a poisoning case where a person was found dead two days after intravenous injection of 0.5 gram of salvarsan. The stomach-contents contained not a trace of arsenic though it was found in other organs. Since the dose of salvarsan may run as high as 1 gram, the body will receive 0.34 gram of arsenic. But elimination of arsenic may continue for months. Not infrequently in legal cases the question may come up whether arsenic found in a cadaver came from salvarsan, or from an inorganic compound containing arsenic. In such cases it should be ascertained whether arsenic can be found in the stomach-contents and further whether salvarsan as such can be detected in the urine. The author has repeatedly examined the urine following intravenous injections of the usual doses of salvarsan or neo-salvarsan. He has found that the resorcinol test, when it appears at all, is entirely negative after a very short time, on the average after 6 hours! Colorimetric determinations have then shown that elimination of salvarsan as such, or in the form of a component (?) permitting formation of azo-dyestuff, is decreasingly small and always amounts to only a few mg. taken as salvarsan! To test for salvarsan, acidify 5–10 cc. of urine with hydrochloric acid, add a few drops of 0.5 per cent. sodium nitrite solution, and gradually add this mixture to a solution of 0.3 gram of resorcinol in about 5 cc. of water, to which 2–3 cc. of 20 per cent. sodium carbonate solution have been added. If the urine contains salvarsan, the resorcinol solution will turn red. Otherwise only a yellow color appears. In cases of sudden death the forensic chemist by means of this reaction can detect salvarsan still present in the urine, unless of course the salvarsan was injected too long ago. Directions given by Abelin[2] for detecting salvarsan in urine are almost identical with those above. He acidifies a measured quantity of urine (about 8 cc.) in a test-tube with 6–8 drops of hydrochloric acid, cools well, adds 3–4 drops of 0.5 per cent. sodium nitrite solution, and pours a few drops of this mixture into about 5–6 cc. of a colorless, alkaline 10 per cent. resorcinol solution. The latter immediately turns red in presence of salvarsan. An especially good method of observing this color is to pour the urine-mixture carefully without shaking upon the resorcinol solution. The reaction-mixture is then allowed to stand and after some time a dark zone appears in the upper liquid. By this method urines that contain no salvarsan, and also free nitrous acid, give only a

[1] G. Müller: Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw. (3) 49 (1915), 48.

[2] L Abelin: A New Method of Detecting Salvarsan. Münch. med. Wochenschr. 1911, page 1002.

yellow color. In case of atoxyl-urine treated in the same manner only an orange color appears.

In the examination of parts of the cadaver of a man who had been treated with salvarsan, Richter[1] was able to detect arsenic only at the site of injection. He extracted the given material with alcohol containing hydrochloric acid. In the liver and spleen he was unable to find arsenic. In six cases of salvarsan poisoning terminating fatally the author obtained different results. He always found arsenic in the liver in appreciable quantities and less in the kidneys and urine, whereas the brain was free. The liver of a man, who died 3 months after the last salvarsan injection, still contained 23.6 mg. of arsenic.

Notwithstanding the fact that elimination of arsenic in the urine has apparently ceased, an appreciable quantity deposited in the organs is still retained in the human body for months. According to Bornstein,[2] after intravenous injection most of the arsenic in organic combination does not circulate freely in the blood but is deposited in the natural storehouses of the body, that is, in the liver, spleen and kidneys. Likewise salvarsan absorbed from the sites of subcutaneous and intramuscular injections does not remain in the blood circulation but is stored in the organs mentioned. The fact is also notable that a considerable quantity of arsenic following intramuscular injection remains for some time in the injected muscle. In the case of a woman who died 36 days after an injection, Fischer and Hoppe[3] found 0.01 gram of arsenic in the muscle. In the case of a man who had been treated with 0.7 gram of salvarsan and had died 3 weeks after injection, Gaebel[4] examined a piece of flesh from the back surrounding the site of injection, consisting of skin, subcutaneous cell-tissue and muscle. The piece of flesh weighed 143 grams and contained in all 0.074 gram of arsenic, corresponding to 0.22 gram of salvarsan. Gaebel states that unquestionably the quantity of arsenic retained in the characteristically colored area of the site of injection was somewhat greater, for the examination did not include the entire region.

Detection in Organic Material.—Stir the material with several times its volume of 96 per cent. alcohol acidified with a few drops of hydrochloric acid. Then digest the mixture for several hours at gentle heat and finally filter. Gradually add absolute alcohol to the syrupy residue left upon evaporation of the filtrate with moderate heat until nothing more is precipitated. Again filter, evaporate the filtrate and dissolve the residue in a little water. Filter the aqueous solution, testing for arsenic by the Reinsch and Gutzeit tests and for salvarsan by the diazo-reaction mentioned above. Two salvarsan urines gave a very distinct reaction by the α-naphthylamine test with the unchanged urine direct.

Approximately Quantitative Estimation of Arsenic in Salvarsan

By the Schneider-Fyfe-Beckurts method, that is, by destroying organic matter with hydrochloric acid and potassium chlorate and

[1] R. Richter: Detection of Salvarsan in Parts of Cadavers. Pharm. Ztg. 56 (1911), 314.

[2] Bornstein: Deutsche med. Wochenschr. 1911, page 112.

[3] Fischer and Hoppe: Münch. med. Wochenschr. 1910, page 1530.

[4] G. O. Gaebel: Salvarsan in the Forensic Detection of Arsenic. Arch. d. Pharm. 249 (1911), 49.

distilling in a stream of hydrochloric acid with addition of ferrous chloride, Gaebel[1] in two determinations obtained results for arsenic agreeing very well with each other but deviating considerably from calculated values. Instead of 33.6 per cent. of arsenic, he found only 29 and 29.5 per cent. Moreover after destroying the organic salvarsan complex in a Kjeldahl flask with nitric and sulphuric acids, he again found only 29.3 per cent. of arsenic.

Neo-Salvarsan and Sodium-Salvarsan

Neo-salvarsan is a yellowish powder of characteristic odor dissolving easily in water with neutral reaction. It is a salvarsan derivative ready for use by simple solution in water and requiring no alkalization nor other treatment as the old salvarsan does. At the same time the activity of neo-salvarsan in correspondingly higher doses is the same as that of salvarsan. Neo-salvarsan is very sensitive to atmospheric air, quickly becoming highly toxic in contact with oxygen. For this reason this preparation is supplied in glass ampullae filled with an indifferent gas and sealed by fusion. Thus protected from oxidative processes this preparation keeps indefinitely. It contains about 20 per cent. of arsenic. In addition to the active arsenical compound, it also contains inorganic salts, especially a large quantity of common salt. With regard to arsenic-content

0.1 gram of Salvarsan corresponds to 0.15 gram of Neo-salvarsan = Dose I
0.2 gram of Salvarsan corresponds to 0.30 gram of Neo-salvarsan = Dose II
0.3 gram of Salvarsan corresponds to 0.45 gram of Neo-salvarsan = Dose III
0.4 gram of Salvarsan corresponds to 0.60 gram of Neo-salvarsan = Dose IV

Only in special cases that are exceptional are individual doses larger than 0.6 gram of neo-salvarsan given to men, or 0.45 gram to women. Neo-salvarsan solutions according to concentration are prepared with sterilized distilled water, or with diluted salt solution (0.4 per cent. at highest), at blood temperature (about 37°). Solutions having too high salt-content are hypertonic and easily cause turbidity. They should not be heated because neo-salvarsan is decomposed. Neo-salvarsan solutions should be used as soon as prepared, since they are oxidized even more easily than solutions of old salvarsan. Neo-salvarsan is applied intravenously, in rare cases intramuscularly.

Preparation.—Formaldehyde-sulphoxylic acid, or methane-sulphinic acid, is allowed to act upon meta-diamino-para-dioxy-arseno-benzene, the mother-substance of salvarsan, and the condensation-product converted into its sodium salt. This is neo-salvarsan. The entire chemical process should be carried out with utmost care and with regard for certain precautions, otherwise substances of very high toxicity may be formed (Report from Höchst Color Works):

$$(HO)(NH_2)C_6H_3.As=As.C_6H_3(ON)NH\overset{\cdots}{H} + \overset{\cdots}{HO}CH_2.SONa =$$
$$H_2O + (HO)(NH_2)C_6H_3.As=As.C_6H_3(OH).NH.CH_2.SONa$$

So neo-salvarsan may be designated as dioxy-diamino-arseno-benzene sodium mono-methane-sulphinate.

Sodium-salvarsan is a fine, golden-yellow powder of characteristic odor easily soluble in water with alkaline reaction. In atmospheric air it undergoes pro-

[1]Loc. cit.

found chemical change attended with very marked increase in toxicity. As a result the preparation takes on a darker or brown color and becomes insoluble in water (Höchst Report). To protect salvarsan-sodium from action of light, it should be filled in glass ampullae *in vacuo*, or sealed. Its arsenic-content like that of neo-salvarsan is about 20 per cent. With regard to arsenic-content

0.1 gram of Salvarsan corresponds to 0.15 gram of Salvarsan-sodium = Dose I
0.3 gram of Salvarsan corresponds to 0.45 gram of Salvarsan-sodium = Dose III
0.5 gram of Salvarsan corresponds to 0.75 gram of Salvarsan-sodium = Dose V
0.6 gram of Salvarsan corresponds to 0.90 gram of Salvarsan-sodium = Dose VI

In corresponding doses the potency of salvarsan-sodium is the same as that of salvarsan. Primarily salvarsan-sodium is employed intravenously but, if a careful technique is observed, it may also be injected intramuscularly or subcutaneously. It is further used rectally and in very small doses intralumbarly.

Preparation.—Salvarsan as a diacid phenol is converted by sodium hydroxide into a disodium salt that may be isolated by means of a suitable precipitating agent. The entire procedure as in the case of neo-salvarsan should be carried out with utmost care and with regard for certain precautions, otherwise substances of very high toxicity may be formed.

Colorimetric Estimation of Neo-Salvarsan and Sodium-Salvarsan in Urine

(Autenreith and Taege[1])

As a di-primary aromatic amine salvarsan and derivatives in therapeutic use may be diazotized by nitrous acid. The diazonium salts formed may then be coupled in alkaline solution with monatomic and polyatomic phenols and thereby converted into azo-dyestuffs. Of the phenols, resorcinol is particularly well adapted for this purpose. Abelin has also recommended it for the qualitative detection of salvarsan in urine. Phloroglucinol is also suitable and has an added advantage over resorcinol that its alkaline solution is more stable and may be kept even for several weeks in dark bottles. Since, however, the phloroglucinol test is not more delicate, on account of the higher cost of the reagent the authors in their experiments have used resorcinol which may be purchased in any drug-store and is readily obtained pure by distillation (Bpt. 276°).

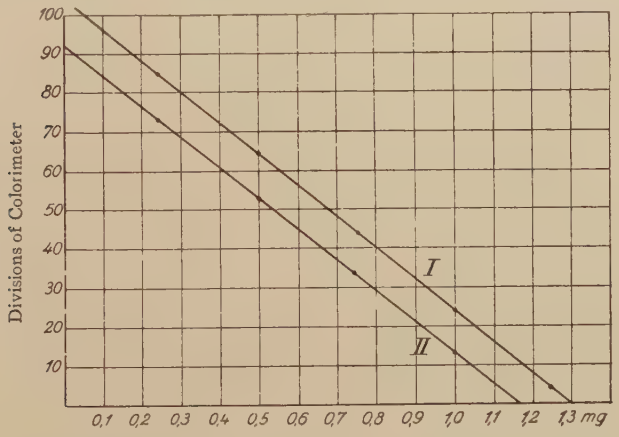
Calibration of the Comparison Wedge

As in all colorimetric determinations made with the Autenrieth-Koenigsberger colorimeter, a comparison-wedge filled with an artificial, colored solution that can be kept without change is required. Such a solution having the same hade as that of the solution under examination is easily prepared by mixing solutions of cobaltous nitrate, ferric salt, copper sulphate and chrome alum. Such a wedge must first be calibrated. Dissolve 0.1 gram of neo-salvarsan in 200 cc. of water that has been previously boiled and cooled. To prevent oxidation, pass hydrogen through the solution at the same time. Also after diazotization excess of nitrous acid should be removed at once with urea, otherwise pure shades of color cannot be obtained with the resorcinol solution. With a capillary pipette

[1] W. Autenrieth and H. Taege: Elimination and Estimation of Salvarsan in Urine. München. mediz. Wochenschr. 1922, page 1479.

exactly measure different quantities of the neo-salvarsan solution, that is, 0.5–2.5 cc. Each time deliver the solution into a small graduate well-cooled with ice, dilute to 5 cc. with water, acidify with 3–4 drops of dilute hydrochloric acid, and finally add 4–5 drops of 0.5 per cent. sodium nitrite solution. After 1 minute fill to the 10 cc. mark with saturated urea solution, shake and at once return the graduate to the ice. Shaking from time to time soon decomposes excess of nitrous acid and a drop of the diazonium solution then colors starch-iodide paper neither blue nor violet. Then add 5 cc. of a mixture, containing 5 cc. of 6 per cent. aqueous resorcinol solution and 3 cc. of saturated sodium carbonate solution (about 20 per cent.), previously prepared and cooled with ice. Shake thoroughly, return to the ice, and after 1–2 minutes repeatedly standardize against the same color strength. (For the full description of the colorimeter, see page 468.) In the calibration of a comparison-wedge the following correlated values were obtained:

Neo-salvarsan solution:	0.5 cc.	1 cc.	1.5 cc.	2 cc.
Water	4.5 cc.	4 cc.	3.5 cc.	3 cc.
Neo-salvarsan	0.25 mg.	0.5 mg.	0.75 mg.	1 mg.
Reading for equal color strength	83/84	62/63	43/44	23/24



Neo-salvarsan—Curve I—in 15 cc. of color-solution or 5 cc. of urine
Sodium-salvarsan—Curve II—in 15 cc. of color-solution or 5 cc. of urine

FIG. 28.

These values are the basis of the above calibration curve of the comparison-wedge. In the same manner the calibration of the comparison-wedge for sodium-salvarsan may be made by dissolving 0.1 gram of this substance in 200 cc. of water.

To estimate these substances in urine, the procedure is the same as that above for the calibration of the wedge, except that 5 cc. of urine are measured instead of neo-salvarsan or sodium-salvarsan. By setting the color solutions in ice they may be kept about 5 minutes. Then a perceptible brown color appears. This may be due to resorcinol, for it does not appear when phloroglucinol is used.

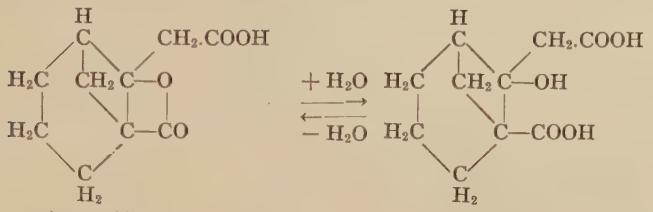
If possible, neo-salvarsan or sodium-salvarsan should be determined in freshly voided urine, since the content of these compounds may rapidly diminish on account of the ease with which they are oxidized even in atmospheric oxygen. In one instance a content of 18.7 mg. of neo-salvarsan in a urine that stood in the air for a day dropped to 11.4 mg. On the basis of a long series of experiments the statement may be made that the quantity of salvarsan derivatives eliminated as such through the kidneys is exceedingly small as compared with that introduced intravenously. After intravenous injection the total quantity of neo-salvarsan, or sodium-salvarsan, eliminated through the kidneys ranges from 0.45–0.6 gram within a limit of 10–27 mg. In fact after the first 2 hours 5.2–19 mg. are eliminated; in the third and fourth hour the quantity of salvarsan eliminated usually drops considerably; and 6 hours after injection hardly a trace of salvarsan can be found in the urine. Expressed in percentages these quantities are always small, usually less than 8 per cent. of the salvarsan being eliminated in the urine. After injection of 0.6 gram = 600 mg. of neo-salvarsan, 29 mg. were eliminated in the urine within 4 hours, that is, not more than 4.6 per cent. of the quantity of salvarsan introduced.

CANTHARIDIN

Cantharidin, $C_{10}H_{12}O_4$, the active vesicating principle of Spanish fly (Lytta vesicatoria), is present to the extent of 0.8–1 per cent. Cantharidin forms colorless, shining, neutral, rhombic leaflets, melting at 218° and subliming at higher temperature in white needles. Boiled with water and also with alcohol, cantharidin is volatile with water or alcohol vapor. It is almost insoluble even in boiling water. Acids, such as tartaric acid, increase its solubility in water, though cantharidin is not a base. It dissolves with difficulty in cold ethyl alcohol (0.03 : 100 at 18°) and in ether (0.011 : 100). Chloroform (1.52 : 100), acetone and acetic ether are its best solvents. It is practically insoluble in petroleum benzine.

Constitution.—Warmed with potassium or sodium hydroxide solution, cantharidin dissolves forming salts of cantharidic acid unknown in the free state. On the other hand, if solutions of these alkali salts are acidified and warmed, cantharidic acid at first set free loses water within the molecule and passes into its inner anhydride, cantharidin, which then separates. Potassium cantharidate, $K_2C_{10}H_{12}O_5.2H_2O$, and sodium cantharidate, $Na_2C_{10}H_{12}O_5.2H_2O$, are well crystallized salts. Cantharidin heated with hydriodic acid at 100°, or with chlorosulphonic acid, $Cl.SO_2.OH$, at 0°, changes into the isomeric cantharic acid, $C_{10}H_{12}O_4$, which is crystalline and a strong monobasic acid. This acid is not a vesicant. Cantharic acid differs from cantharidin in being water-soluble. Acetyl chloride causes cantharic acid to undergo rearrangement and form the isomeric isocantharidin. The close relationship between cantharidin and o-xylene is shown by the fact that cantharic acid heated with caustic lime gives a dihydro-o-xylene, C_8H_{12}, the so-called cantharene, and cantharidin under the same conditions gives

with phosphorus pentachloride o-xylene. Upon the basis of this chemical behavior H. Meyer has assigned the following structural formulae to cantharidin and cantharidic acid:



Cantharidin Cantharidic acid

Cantharidin according to H. Meyer is a β-lactone and at the same time a monobasic acid. If the lactone ring is broken by caustic alkalies, the well crystallized alkali salts of dibasic cantharidic acid are formed. If these alkali salts are acidified with hydrochloric or sulphuric acid, at first free cantharidic acid is formed but this at once passes into cantharidin, its inner anhydride.

Toxic Action.—Cantharidin produces severe, local inflammation at the site of application, upon the outer skin or in the intestinal tract; at a distance it causes inflammation of the ureters and genital organs. Cantharidin acts upon the skin as a vesicant. In addition, a striking leucocytosis appears, as well as severe, acute inflammation of the kidneys, cantharidin nephritis. Although scarcely soluble in water, cantharidin is absorbed from the stomach as well as from the skin when intact. Before being absorbed from the latter, it causes inflammation and produces blisters.

Elimination in the urine of cantharidin as a salt of cantharidic acid starts in very soon. It also takes place through the intestinal glands. Consequently a secondary effect is intestinal irritation, even when the poison has not been taken by the mouth.

Cantharidin is a very powerful poison, for even 10 mg. may have a highly toxic action. As a rule death ensues after 1–5 days. In case of recovery frequently chronic inflammation of the kidneys persists.

Detection of Cantharidin in Organs, Blood and Urine

Render the material, stomach and intestines with contents, vomitus, blood, parts of liver, kidneys, brain, etc., strongly alkaline with dilute potassium hydroxide solution (1 gram of KOH in 15 cc. of water) and then warm the mixture until thoroughly uniform. If present, cantharidin passes into solution as potassium cantharidate. The mixture should show an alkaline reaction, otherwise more potassium hydroxide solution must be added. When cold, shake with chloroform to remove unsaponified fat and other impurities. Draw off the aqueous alkaline solution and acidify with dilute sulphuric acid. Add 5 times the volume of alcohol and reflux for 30 minutes to convert cantharidic acid into cantharidin. Filter

when cold, distil[1] alcohol from the filtrate, and extract the unfiltered residue with chloroform. Evaporate the filtered chloroform extract, or distil the solvent, and use a portion of the residue for the physiological test. If this residue does not contain sufficient fat, dissolve in a few drops of hot almond oil. Bind a cloth saturated with this solution upon the upper arm or breast by adhesive tape. Cantharidin reddens the skin, sometimes raising pustules and blisters. Even 0.14 mg. is said to produce blisters. Salts of cantharidic acid also have a vesicating action.

If the physiological test is distinct or even strongly positive, J. Gadamer recommends attempting the preparation of pure cantharidin by the following method. Triturate the remainder of the residue from the above chloroform extract with petroleum ether, which dissolves no cantharidin, and then with a little cold alcohol, which dissolves scarcely any. What remains undissolved is recrystallized from acetic ether. The residue freed from fat by petroleum ether may be converted by warming with as little potassium hydroxide solution as possible into potassium cantharidate which owing to ready diffusibility can easily be purified by dialysis. The liquid outside the dialyzer may be treated with a little sulphuric acid and boiled for a very short time. This will yield almost pure cantharidin to chloroform. To recognize cantharidin, use may be made of the melting-point 218°, insolubility in water, solubility in potassium and sodium hydroxide solution, especially in presence of alcohol.

To examine urine or other liquids for cantharidin, acidify with dilute sulphuric acid and extract with chloroform which dissolves cantharidin. If urine contains albumin, first precipitate that by alcohol.

Notes.—Detection of cantharidin to be positive and free from objection cannot depend upon the physiological test alone, for pepper and paprika contain substances producing physiological results similar to those given by cantharidin. The article upon this matter in J. Gadamer's "Chemical Toxicology" (1911) should be consulted.

ARTIFICIAL HYPNOTICS AND SEDATIVES

In his "Textbook of Pharmacology" (1918), Tappeiner divides hypnotics into two groups. The first embraces alkaloids that have

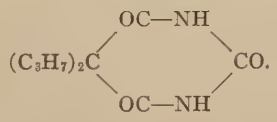
[1] Since cantharidin is volatile with the vapor of alcohol, evaporate the distillate after rendering it alkaline with a few drops of sodium hydroxide solution. Then acidify the residue with dilute sulphuric acid, heat to boiling, and extract with chloroform any cantharidin that may be present.

hypnotic properties; the second hypnotics of the aliphatic series. The former may also be designated as natural, the latter as artificial hypnotics. With few exceptions, artificial hypnotics belong to the aliphatic series. The author believes that a classification of artificial hypnotics suggested by Nolte[1] deserves consideration, for it arranges them according to chemical constitution in one of the following four sub-groups:

1. Derivatives of alcohol and related compounds.
2. Hypnotics belonging to the urea group.
3. Sulphonals that are derivatives of mercaptans.
4. Hypnotics that cannot be classified in the preceding groups.

A fifth group made up of combinations and transitions from one into the other groups might be added. The hypnotic effect of an agent, according to Bürgi, may be increased by combining two narcotics not belonging to the same group. In this case not only is the action of the two components additive but sometimes potency is increased. From this view-point has come into existence codeonal, a mixture of 2 parts of veronal-codeine (codeine diethyl-barbiturate) and 15 parts of medinal. This combination has proved of value in insomnia due to bronchitis. In general a hypnotic should act quickly and produce an effect of sufficient duration. Substances readily soluble in water, that are uniformly distributed in the stomach and absorbed in the intestines, will best meet these conditions. But above everything else hypnotics should not produce dangerous secondary effects upon circulation, respiration and metabolism. To render soluble various hypnotics like veronal and luminal, that dissolve in water with difficulty, especially for injection, convert them into sodium salts.

Proponal



Proponal, dipropyl-barbituric acid, or dipropyl-malonyl-urea, belongs to the same class of compounds as veronal (see page 137). It is a white, crystalline powder, melting at 145° and having a faintly bitter taste. In cold water it dissolves with great difficulty, more

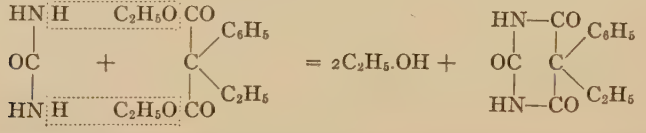
[1] E. Nolte: Newer Hypnotics, Constitution and Action. Zentralblatt für Pharmazie 17 (1921), 336.

easily in hot, and readily in alcohol, ether, chloroform, and aqueous solutions of caustic alkalies. Acidification of alkaline solutions not too dilute again precipitates proponal.

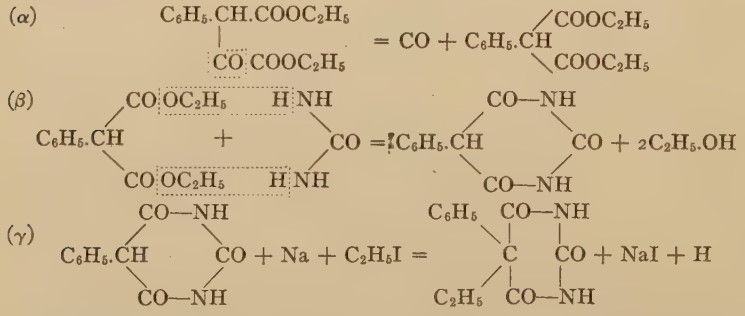
Physiological Properties.—Dialkyl-barbituric acids like disulphones show a relationship between chemical structure and physiological action. The dimethyl-compound has hardly any soporific action; the diethyl-compound, veronal, very marked action; and the dipropyl-compound, proponal, maximum action. The relative doses 0.5 gram of veronal = 0.25–0.3 gram of proponal about correspond to the difference in action. In therapeutic doses proponal produces sleep within 15–40 minutes. On the average, duration of sleep is 7 hours. In addition to being a hypnotic, proponal acts distinctly in allaying pain. Days after its use a feeling of exhaustion, stupor, vertigo and nausea may appear. Among secondary effects may be mentioned decrease in blood-pressure; on the other hand, accelerated heart action and increase of blood-pressure.

Luminal

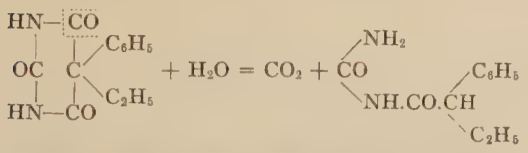
Luminal, phenyl-ethyl-malonyl-urea, or CC-phenyl-ethyl-barbituric acid, is formed by the action of urea upon CC-phenyl-ethyl-malonic ester:



Preparation.—Phenyl-oxalyl-acetic ester loses carbon monoxide on distillation and is converted into monophenyl-malonyl-diethyl ester (α) which with urea forms phenyl-barbituric acid (β) and then luminal on ethylation with sodium and ethyl iodide(γ):



From hot water luminal crystallizes in white, inodorous leaflets melting at 170–171° and having a faintly bitter taste. It is almost insoluble in cold water but freely soluble in organic solvents and dilute aqueous alkaline solutions. By continued action of alkalies luminal is decomposed into carbon dioxide and phenyl ethyl-acetyl-urea:



Action of Luminal.—Luminal has a powerful hypnotic action. Koenig[1] has obtained very good results with luminal in tablet form and subcutaneously in hundreds of cases as a hypnotic and sedative, especially with women, and has observed exanthema in only two cases. Others have also recommended luminal as a good sedative and hypnotic and van Reysschoot (Bulletin de la Société de Médecine mentale de Belgique 1913) has pronounced it the best of our drugs as an anti-epileptic. Luminal, according to von Klebelsberg,[2] has a very favorable action upon epilepsy. Apparently it may even cure the disease in cases where bromine proved ineffective. Secondary effects, though not observed very frequently, call for special care on account of their severity.

Secondary Effects.—After administration of 4 luminal tablets of 0.3 gram each, Müller[3] observed severe vomiting, disturbances of speech, unsteady gait, headache, vertigo, and disturbances of vision in conjunction with narcotic action. Itching exanthema of the skin appeared 8 days after this dose of luminal. Haug[4] previously reported two cases of luminal poisoning. In one case, following administration of 0.1 gram of luminal 3 times daily, suddenly scarlet exanthema with high fever and bloody, mucous diarrhoea appeared 4 weeks after the beginning of treatment. After stopping the drug recovery followed. In the second case high fever and scarlet exanthema together with copious mucous diarrhoea without blood, also albuminuria and severe interference with the sensorium, appeared after using luminal for 11 days. In this case too these effects disappeared when use of luminal was stopped.

Detection of Luminal.—Add luminal to urine and then examine this artificial luminal-urine by the Stas-Otto process. Evaporate the urine after acidification with tartaric acid. Take up the residue in alcohol, filter and evaporate the filtrate. Dissolve the residue in water and extract with ether. By this method 75 per cent. of the luminal taken may be recovered in the urine. Following administration, however, of 0.3–0.6 gram of luminal, none could be found in the urine (Nolte[5]). Consequently the supposition is that even

[1] H. Koenig: Critical Notes upon Luminal. Berliner klinische Wochenschrift 1912, No. 40.

[2] v. Klebelsberg: Experiences with Luminal. Psychiatr.-Neurol. Wochenschrift 15 (1913), No. 34.

[3] W. Müller: Secondary Effects from Luminal. Therapeutische Monatshefte 34 (1920), 79.

[4] W. Haug: Two Cases of Luminal Poisoning. Münchener medizinische Wochenschrift 66 (1919), 1494.

[5] E. Nolte: Pharmaz. Zeitung 65 (1920), 320.

after large doses, or protracted use of luminal, it cannot be detected either in parts of the cadaver or in urine. Since presence of a phenyl in place of an ethyl group is the only difference between luminal and veronal, the former appears to be the deciding factor in bringing about the complete decomposition of luminal in the animal organism. Before testing for luminal, recrystallize the given substance from hot water with use of animal charcoal and then take its melting-point. Use may also be made of its solubility in dilute alkalies, sodium carbonate solution and ammonia and of the fact that it is reprecipitated upon acidifying these solutions, if not too dilute.

Sodium-Veronal (also called **Medinal**).—This substance is the mono-sodium salt of diethyl-barbituric acid. It is a white, crystalline powder, having rather a bitter and somewhat caustic taste and dissolving in 5 parts of cold water. Sodium-veronal appears in commerce as the substance itself and as 0.5 gram tablets.

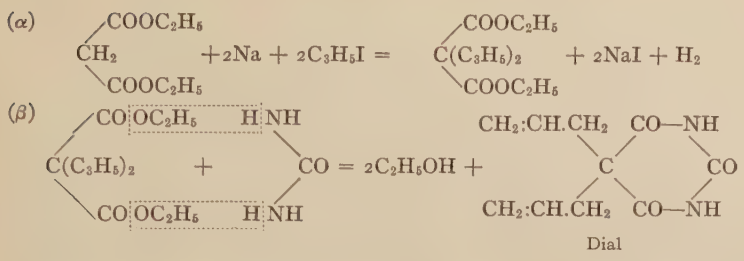
Physiological Action.—Therapeutic doses of 0.5 gram of veronal taken at a time produce in man within 0.5–0.75 of an hour sleep that as a rule is like physiological sleep and lasts 7–8 hours. Appearance of action depends upon the time it takes veronal to reach the intestinal tract where it is absorbed as alkali salt. When in solution, especially if the stomach is empty, veronal acts before it leaves the stomach, that is, more rapidly than when undissolved. On this account sodium-veronal is to be preferred to veronal itself, as it is freely soluble in water and consequently reaches the intestine quickly enough even though it is used undissolved.

Sodium-Luminal.—This compound is the mono-sodium salt of phenyl-ethyl-barbituric acid and is obtained by dissolving luminal (1 mol.) in sodium hydroxide solution (1 mol. NaOH) and evaporating the solution to dryness. It is a white, crystalline, hygroscopic powder freely soluble in water and especially adapted for subcutaneous use. Upon long standing, or after prolonged boiling of solutions of sodium-luminal, carbon dioxide is set free and phenyl-ethyl-acetyl-urea having only a feeble hypnotic action is precipitated. For this reason solutions of this compound should not be kept too long.

DIAL

Dial or diallyl-barbituric acid, that is, diallyl-malonyl-urea, is a hypnotic belonging like veronal, proponal and luminal to the urea-group.

Preparation.—The diethyl ester of diallyl-malonic acid is obtained by the action of sodium and allyl iodide upon diethyl-malonate (α). This product treated with urea gives dial (β):



Dial

Physiological Action.—Injections of dial, according to Castaldi,[1] produce sleep rather rapidly in frogs and rabbits, as this drug does in man when taken by the mouth. A relatively small dose of dial, as compared with that of other hypnotics of the urea-group, produces an effect. This is ascribed to the presence of allyl-groups. In rabbits, following intravenous administration, an accelerating effect upon the motor heart-ganglia appears. On account of its action upon the heart and the ease with which man becomes habituated to dial, this compound has few advantages over other malonyl-urea derivatives having similar properties. Occasionally headache and vertigo appear after use of dial.

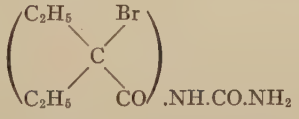
Didial is a combination of dial and dionine, or ethyl-morphine. It is used especially to produce twilight sleep. According to Bürgi, hypnotic action may be increased by combining two narcotics not belonging to the same group. In this case not only is an added effect from the two components obtained but sometimes action is more potent.

ORGANIC HYPNOTICS CONTAINING BROMINE

(Adaline, Bromural, Neuronal, Diogenal)

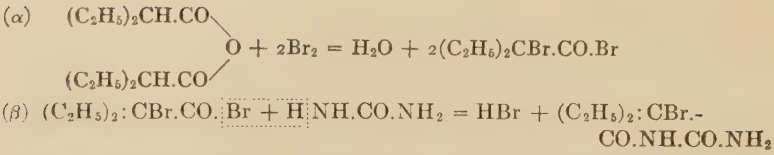
ADALINE

Adaline is the medico-pharmaceutical name given to bromo-diethyl-acetyl-urea:

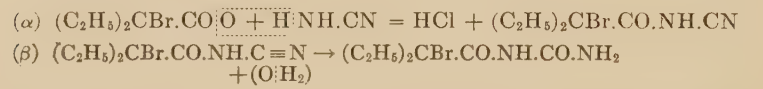


Preparation.—Bromo-diethyl-acetyl-bromide, formed by the action of bromine upon tetra-ethyl-acetic anhydride, is converted into adaline by urea:

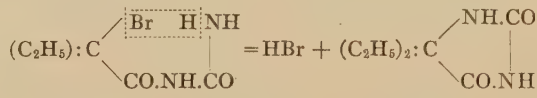
[1] L. Castaldi: Pharmacological Properties of Diallyl-malonyl-urea. Arch. di Farmakol. sperim. 19 (1915), 289.



It may also be prepared by the addition of a molecule of water to bromo-diethyl-acetyl-cyanamide, formed by the action of cyanamide upon bromo-diethyl-acetyl chloride:



Adaline is a colorless, inodorous and nearly tasteless, crystalline powder melting, but not sharply, at 114–118°. In cold water it dissolves to the extent of only 0.05 per cent. but is more readily soluble hot; in alcohol, benzene and acetone it is freely soluble. When boiled with water, it is decomposed and also by sodium hydroxide solution. From warm alcohol with water it crystallizes in white needles melting at 117–118°. After heating with water, Br′-ion can be detected with silver nitrate. With aqueous potassium hydroxide solution it evolves ammonia; with alcoholic potassium hydroxide solution it gives ammonia, potassium bromide and potassium cyanide. Upon longer heating with water, but best with pyridine, apparently α-diethyl-hydantoin is formed, crystallizing from benzene in needles melting at 182–183° (Rosenmund and Herrmann[1])



Physiological Properties.—Adaline is a sedative having a feeble hypnotic action. Upon rabbits according to Filippi,[2] it has a poisonous action in doses of 0.7 gram per kilogram of body-weight; produces narcoses with 0.64 gram; and light but quite lasting sleep with 0.53 gram. It is said to have no injurious action upon the heart. Adaline does not pass into solution until it reaches the alkaline intestinal juice. Elimination of only a small part unchanged takes place through the urine. Most of it is present in the urine as a brominated acid, possibly as diethyl-bromo-acetic acid, $(C_2H_5)_2$: CBr.COOH(?). The acid isolated from urine melts at about 87°. Bromine in inorganic combination is split out from adaline

[1] K. W. Rosenmund and F. Herrmann: Contribution to the Knowledge of Adaline. Berichte d. Deutsch. pharmaz. Ges. 22 (1912), 86.

[2] E. Filippi: Pharmacological Properties of Adaline. Arch. di Farmakolog. sperim. 12 (1911), 283.

only after administration of toxic doses. According to Airila,[1] the lethal dose for rabbits is 0.7 gram per kilogram of body-weight.

BROMURAL

Bromural is the commercial name of the hypnotic, α-bromo-isovaleryl-urea, $(CH_3)_2CH.CHBr.CO.NH.CO.NH_2$.

Preparation.—This compound may be prepared by the action of urea upon α-bromo-isovaleryl chloride or α-bromo-isovaleryl bromide:

$$(CH_3)_2CH.CH_2Br.CO\,Cl + H\,NH.CO.NH_2 = HCl + (CH_3)_2CH.CHBr.CO.NH.CO.NH_2$$

Bromural crystallizes from toluene in leaflets melting at about 149° (Knoll). According to Zernik,[2] bromural, after repeated solution in a little warm alcohol and precipitation with water, forms white needles melting constant at 154° with previous softening. Though but very slightly soluble in cold water, it dissolves easily in hot water, alcohol, ether, and aqueous alkalies. At a higher temperature bromural can be sublimed. As an acid amide it can be hydrolyzed by acids and alkalies. Boiled with nitric acid, or 50 per cent. sulphuric acid, bromural undergoes partial decomposition. The odor of valerianic acid is apparent but bromine is not split out. Partial decomposition is also caused by boiling with 10 per cent. sodium hydroxide solution, whereby ammonia is evolved and bromine is split out. If bromural (3 grams) is boiled under reflux for 3 hours with 0.5 n-alcoholic potassium hydroxide solution (40 cc.), a mixture of potassium bromide and cyanide separates. At the same time isopropyl-hydantoin is formed. This compound crystallizes from water in compact white crystals melting at 216–217°:

$$(CH_3)_2CH.CH.CO.NH.CO \;(Br \;\; H\;NH) = HBr + (CH_3)_2CH.CH\begin{cases} CO\text{—}NH \\ NH\text{—}CO \end{cases}$$

Isopropyl-hydantoin is very slightly soluble in cold water, more readily in hot water and alcohol, and only slightly in ether. Aqueous alkalies also dissolve it, causing the appearance of a faint red color and evolution of ammonia. Boiled with dilute sulphuric acid, it separates in oily drops soluble in ether.

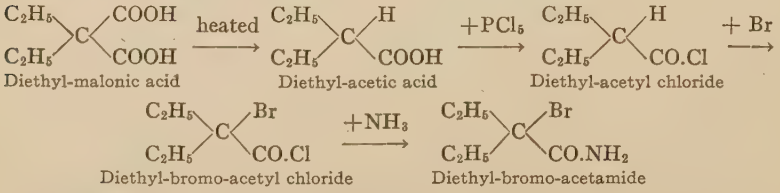
[1] Y. Airila: Experimental Investigations upon Bromural and Adaline. Skand. Archiv f. Physiologie 28 (1913), 193.

[2] F. Zernik: Bromural. Apotheker-Zeitung 22 (1907), 960.

Physiological Action.—According to Airila (see Adaline), the two bromine-substituted acyl-ureas, bromural and adaline, have the same general action upon rabbits but that of adaline is somewhat stronger. The lethal dose of bromural for a rabbit is 1.0 gram per kilogram of body weight. In bromural poisoning the chief symptoms are: paresis[1] of the posterior extremities after 6–40 minutes; 20–50 minutes later, diminution of sensitiveness to pain and of the reflexes, increase of the secretory processes, tracheal rattling, trembling, decrease of respiration, lowering of temperature. After the lethal dose, death ensues in 5–24 hours; after smaller doses, recovery takes place in 5–36 hours. The main effect from bromural, as well as from adaline, is enlargement of the blood-vessels resulting in a marked lowering of blood-pressure. Frequency of breathing is strongly retarded due probably to the direct action of bromural upon the respiratory centre. On the contrary, bromural does not have an injurious action upon the heart.

NEURONAL

Diethyl-bromo-acetamide, $(C_2H_5)_2CBr.CO.NH_2$, is known as neuronal. The equations showing its preparation are:



Neuronal is a white, crystalline powder melting at 66–67°, having an odor faintly suggesting camphor and a burning, slightly bitter taste. In cold water it dissolves 1:120 and is easily soluble in alcohol, ether, chloroform, and fatty oils. Neuronal in aqueous solution reacts acid and upon boiling is decomposed into hydrobromic acid and α-ethyl-crotonamide (Mannich and Zernik[2]):

$$(C_2H_5)_2CBr.CO.NH_2 = HBr + CH_3.CH:C(C_2H_5).CO.NH_2$$

When boiled with sodium hydroxide solution, neuronal is decomposed into hydrobromic acid, hydrocyanic acid and diethyl-ketone:

$$(C_2H_5)_2CBr.CO.NH_2 + 2NaOH = NaBr + NaCN + (C_2H_5)_2CO + 2H_2O,$$

and partly into sodium bromide and diethyl-glycolamide:

$$(C_2H_5)_2CBr.CO.NH_2 + NaOH = NaBr + (C_2H_5)_2C(OH).CO.NH_2$$

In doses of 0.5–2 grams, neuronal acts as a hypnotic and sedative. At the present time fatal poisonings from neuronal are unknown.

[1] Paresis means a slight paralysis.

[2] C. Mannich and F. Zernik: Contribution to the Knowledge of Neuronal. Archiv. d. Pharmazie 246 (1908), 178.

Hydrocyanic acid is split off from neuronal even in the cold and with very dilute alkali. Apparently such a cleavage does not take place in the human organism. Following administration of neuronal, bromine can be detected in the urine even after 14 days and longer.

Detection of Neuronal

The following reactions may be used for the detection of neuronal (Zernik[1]):

Like all organic substances containing bromine, neuronal imparts a blue-green color to a colorless Bunsen flame when burned upon a carefully oxidized copper wire.

If 0.2 gram of neuronal is boiled for a few minutes with 0.1 gram of mercuric oxide and 5 cc. of water, the hot filtered solution upon cooling deposits a white precipitate of mercury-neuronal. Addition of a few drops of potassium iodide solution causes the appearance of a light yellow, voluminous precipitate that gradually becomes crystalline and scarlet-red upon standing.

If 0.1 gram of neuronal is dissolved by boiling in 1 cc. of sodium hydroxide solution and 4 cc. of water, the odor of diethyl-ketone appears. Hydrocyanic acid may be detected in the solution by the Prussian blue reaction. After saturation with dilute sulphuric acid, hydrobromic acid may be detected with potassium permanganate solution and chloroform by the bromine coloration of the latter.

Distribution of Adaline, Bromural and Neuronal in the Organism

To establish distribution of a narcotic between the brain on the one hand and the rest of the body on the other, Gensler[2] took into consideration for the body only the protoplasm actually functioning and susceptible to narcosis. Consequently the weight of the skin, bones, contents of the gastro-intestinal tract and bladder was subtracted from the weight of the body. To determine hypnotic (adaline, bromural, neuronal) in the brain, the latter was first dried upon the water-bath, finely comminuted, mixed with 55 per cent. of its weight of anhydrous disodium phosphate (Na_2HPO_4), completely dried in desiccator, and then extracted for 8 hours in a Soxhlet with ether. The solvent was distilled off and the residue, ashed after addition of a little potassium hydroxide, examined for bromine. The colorimetric method of estimating bromine (see page 298) may also be used. The relation between the total quantity of hypnotic absorbed and that

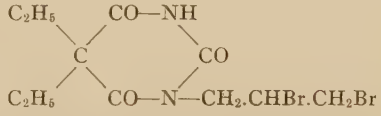
[1] F. Zernik: New Drugs for the Year 1904. Ber. d. Deutsch. pharm. Ges. 15 (1905), 6 and Apotheker-Zeitung 1904, 873.

[2] P. Gensler: Distribution of Neuronal, Bromural and Adaline in the Organism. Archiv. f. experim. Pathol. u. Therapie 79 (1915), 42.

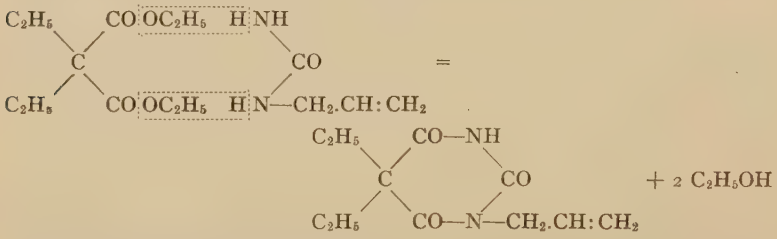
in the brain appears to be quite constant. In case of neuronal, the average percentage in the brain was 1.4 of that absorbed; bromural 1.7 per cent.; and adaline 1.1 per cent. Deviations from these mean values are accounted for by different absolute weights of the brains. Although by far the greater part of the narcotic is distributed throughout the rest of the body and in the blood, the brain relatively has the greatest capacity of absorbing the hypnotics examined (adaline, bromural, neuronal). Upon the average 100 grams of brain contain almost twice as much of the given hypnotic as do 100 grams of all the rest of the protoplasm, blood included. The absorbed hypnotic in acting upon the brain undergoes no decomposition. To produce sleep of the same depth with the weaker hypnotic, more of it must be given and the brain contains a correspondingly greater fraction.

DIOGENAL

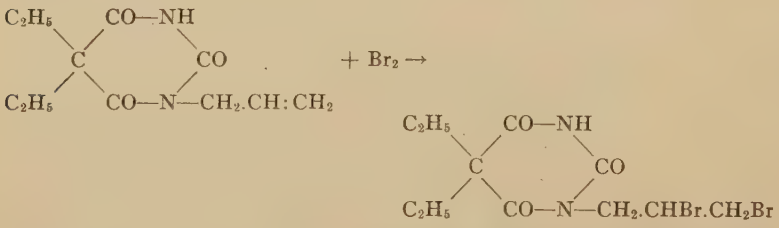
Diogenal is dibromo-propyl-diethyl-malonyl-urea, or dibromo-propyl-diethyl-barbituric acid:



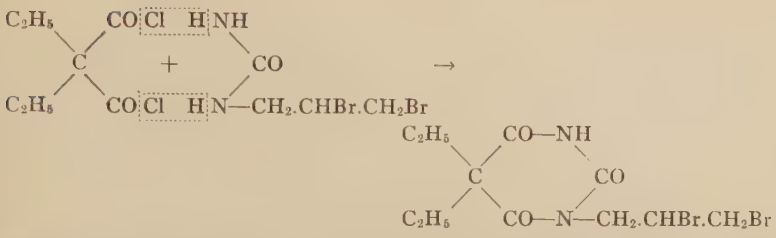
Preparation.—Mono-allyl-urea condensed with the diethyl-ester of diethyl-malonic acid gives mono-allyl-diethyl barbituric acid:



When dissolved in glacial acetic acid, this compound takes up 2 atoms of bromine and forms dibromo-propyl-dethyl-barbituric acid = diogenal:



Diogenal is also formed by condensing dibromo-propyl-urea with diethyl-malonyl chloride:

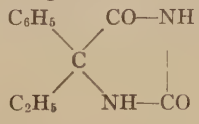


Diogenal appears as a white, crystalline powder, or in colorless needles, having a rather bitter taste and melting at 124–126°. It is obtained in the latter form by precipitating it from solution in hot toluene with petroleum ether, or by crystallization from cold alcohol. It is almost insoluble in water, readily soluble in alcohol and ether, and also in dilute sodium carbonate solution.

Pharmacological Properties.—Diogenal contains the diethyl-barbituric acid radical, of so much value in medicine because of its narcotic action. In the side-chain attached to nitrogen is also halogen alkyl that manifests, especially in bromine derivatives, a powerful sedative action characteristic of organic bromine derivatives. Consequently diogenal closely resembles veronal in action. Introduction of the dibromo-propyl group into the veronal molecule produces the veronal action in milder form. The sedative character and influence of veronal, combined with the action of the bromine component, becomes more prominent, whereas the characteristic hypnotic effect is less marked. Diogenal is only slightly toxic in action. In case of warm-blooded animals its general action is analogous to that of veronal but considerably weaker. In a rabbit 0.25 gram of veronal taken internally as a rule produces sleep and 1 gram death; but 1 gram of diogenal produces sleep and not until more than 3 grams have been taken does deep coma sometimes ending in death appear. Experiments upon animals have also shown that more frequent administration of even larger doses of diogenal cause neither functional nor organic disturbance. Blood and urine remain normal as well as the pulse and blood-pressure. The bromine in diogenal is eliminated only gradually, a large part being retained. This presents the possibility of accumulation of bromine in the organism and of effects arising from the action of this halogen. Diogenal is stable in presence of dilute hydrochloric acid as it occurs in the gastric juice but slowly dissolves in the alkaline intestinal juice. Hardly any deleterious secondary effects have been observed even after prolonged use of diogenal. And up to the present time no indication has been given of cumulative action or chronic poisoning, even following use of diogenal for a month and longer.

NIRVANOL

Nirvanol is the name given to γ,γ-phenyl-ethyl-hydantoin,



Preparation.—Phenyl-ethyl-acetamide, formed by the action of an ethyl haloid upon phenyl-cyano-acetamide, is converted into γ,γ-phenyl-ethyl-hydantoin by means of hypohalogenites:

Nirvanol crystallizes in colorless, inodorous leaflets having hardly any taste. Its solubility in cold water is 1:1600; in hot water 1:110; in alcohol 1:20; and in ether 1:200. Like veronal and luminal it dissolves easily in sodium hydroxide solution forming a sodium salt. The hot saturated aqueous solution reddens litmus paper faintly. It melts at 199–200°. The sodium salt of nirvanol dissolves easily in water and its solution has a bitter-sweet taste.

Reactions for Detection of Nirvanol.—1. If 0.1 gram of nirvanol is heated with 0.5 gram of calcium oxide, the mass takes on a violet-red color, evolves ammonia, and develops the odor of propiophenone.

2. A solution of 0.1 gram of nirvanol in 2 cc. of sulphuric acid is colored red upon addition of 5 drops of sodium nitrite solution.

Therapeutic Use and Toxic Action of Nirvanol.—In hypnotic action nirvanol stands about midway between veronal and medinal. In cases of mild insomnia, especially in neurasthenics, the dose is 0.25–0.3 gram; in more stubborn cases 0.5–1.0 gram. As with veronal, sleep follows after about 2 hours. Medicinal doses do not cause weakening of the pulse, loss of appetite, headache or nausea (Tilling[1]). Only gradual increase of lassitude indicates a cumulative effect of nirvanol. Following administration of nirvanol, no effect upon the centres controlling the heart and respiration is noticed. Nor is any evidence of kidney irritation given. Elimination of urine is normal and it is free from albumin and sugar. The body temperature is not lowered by nirvanol as it is in poisoning by veronal. On the contrary, children show several tenths of a degree increase of fever the same day they take the drug. But the fever drops the day after and shows a tendency to decrease further. The same thing occurs with luminal. Appearance of an exanthema presents an additional analogy to luminal. Curschmann[2] is probably the first to describe a severe case of nirvanol poisoning. In all the patient took 7 grams of nirvanol in 2.5 days. He was very sleepy, slept much both day and night but woke spontaneously between times, ate little, did not vomit, but was very thirsty. The buoyancy of his spirits and the absence of any unpleasant feeling of illness were quite noticeable. At the same time he was somewhat uncertain as to time and place of the most recent events and easily

[1] F. Tilling: Experiences with the New Hypnotic "Nirvanol." Therapeutische Monatshefte 32 (1918), 422.

[2] Curschmann: Therapeutic and Toxic Action of Nirvanol. Therapeutische Monatshefte 32 (1918), 53.

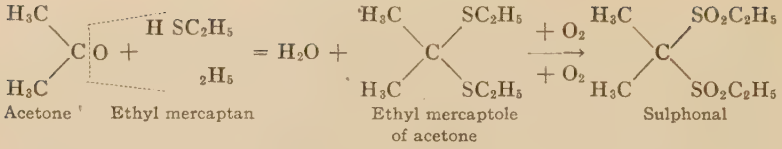
became delirious. He also complained of double vision. His pupils were very much contracted and reacted sluggishly. Respiration was regular, 14–18 to the minute, and pulse 76 to the minute, regular, quite full and firm. Temperature did not increase. Urine contained neither albumin nor sugar. During the entire period of intoxication vomiting did not occur. Curschmann emphasizes especially the fact that during this severe nirvanol poisoning lasting 2.5 days no serious disturbances of circulation and respiration appeared in spite of the large doses of the drug. Although Curschmann lists nirvanol with the more harmless hypnotics, such as adaline and bromural, and not among the "dangerous remedies," other clinicians advise caution because of severe secondary effects that may arise from nirvanol poisoning. Up to the present time have been observed: facial oedema, increase of temperature and cutaneous eruptions, often accompanied by feelings of severe illness. After three doses of nirvanol of 0.5 gram each on three succeeding days, fever and appearance of exanthema were observed. Brünecke gives the following brief summary of his opinion of nirvanol as a hypnotic: "Nirvanol has certain advantages over other hypnotics but doses over 1.0 gram should not be given, and administration after 3–4 days should be stopped." Acetyl-nirvanol, recently recommended as a hypnotic, is said to be without the secondary effects of nirvanol.

SULPHONAL

Sulphonal, $(CH_3)_2C(SO_2C_2H_5)_2$, crystallizes in colorless, inodorous and tasteless prisms, melting at 125–126° and distilling with slight decomposition at 300°. It dissolves at 15° in 425 parts of water, at 37° (blood temperature) in 220 parts, and at 100° in 8 parts. It is also soluble at 15° in 65 parts of alcohol (90 per cent.), in 80 parts of ether, and in 3.5 parts of chloroform. It is volatile with steam. At ordinary pressure it begins to evaporate at about 66° and the vapor has a peculiar odor (Falck[1]). Solutions of sulphonal do not affect litmus paper. Especially characteristic of this compound is its great stability in presence of chemical agents. Halogens, halogen acids, alkaline hydroxides and carbonates, concentrated sulphuric and nitric acids are without action in the cold.

Preparation.—Condensation of ethyl mercaptan (2 molecules) with acetone (1 molecule) by means of dry hydrogen chloride gas, or concentrated sulphuric acid, results in formation of the ethyl-mercaptole of acetone. The latter, shaken with a saturated solution of potassium permanganate in presence of dilute sulphuric acid, undergoes oxidation with formation of dimethyl-methane-diethyl-sulphone = sulphonal:

[1] A. Falck: Contribution to the Knowledge of Sulphonal. Pharm. Zentralbl. 1919, No. 36.



Acetone | Ethyl mercaptan | Ethyl mercaptole of acetone | Sulphonal

Detection of Sulphonal

Ether, or better chloroform, extracts sulphonal from acid, neutral and alkaline solutions. Test the residue left upon evaporating these solutions as follows:

1. **Melting-point Test.**—Absolutely pure sulphonal melts at 125–126°. Crystallization from boiling water with the use of a little bone-black easily gives a pure product. Mix the given crystals with what is known to be pure sulphonal. If the substance in question is sulphonal, this mixture should also melt at 125–126°.

2. **Reduction Test.**—Sulphonal heated in a test-tube with powdered wood charcoal gives the characteristic mercaptan odor.

3. **Detection of Sulphur.** (a) **With Sodium.**—Fusion of sulphonal in a dry test-tube with a little metallic sodium gives sodium sulphide. Dissolve the cold melt cautiously (unaltered metallic sodium!) in a little water, filter and test the filtrate with sodium nitroprusside solution for sulphide (see page 305).

(b) **With Potassium Cyanide.**—Fuse 1 part of sulphonal with about 2 parts of pure potassium cyanide in a dry test-tube. Note the penetrating mercaptan odor. Potassium sulphocyanate is also a product of the reaction. An aqueous solution of the melt, acidified with dilute hydrochloric acid, becomes deep red with 1–2 drops of ferric chloride solution.

(c) **With Powdered Iron.**—Sulphonal heated with pure powdered iron free from sulphur gives a garlic-like odor. Add hydrochloric acid to the residue. Hydrogen sulphide evolved blackens lead acetate paper.

In suspected sulphonal poisoning, examine the contents of stomach and intestines, brain, parts of organs, and especially the urine. Even when parts of the cadaver have entered into an advanced stage of putrefaction, detection of sulphonal is possible, since this substance is very resistant to putrefaction.

Physiological Action.—Sulphonal in doses of 1–2 grams, especially when taken in powder form with enough of a warm liquid to bring most of it into solution, produces sleep in 1–3 hours. Because of its slight solubility sulphonal is slowly absorbed. Therefore its hypnotic action is less rapid than that of many of the

other hypnotics. Owing to conditions unfavorable to its decomposition and elimination, sulphonal is retained by the organism. Thus it produces cumulative effects. When administered for some time without interruption and in considerable doses, much of the drug may collect in the body. This also accounts for its prolonged action, for a single dose of sulphonal, or trional, frequently produces sleep on the second night. This delayed action proves that even after 24 hours these two hypnotics circulate in the body in active form. Consequently sulphonal and trional are indicated as hypnotics, especially in cases where prolonged action is desired. In the usual medicinal doses sulphonal has no injurious action upon the circulation of the blood, respiration and processes of digestion. After very large doses, and also after uninterrupted use of smaller quantities of sulphonal, or even trional, symptoms of poisoning appear. These become manifest from an action upon the digestive system, metabolism, and central nervous system. Symptoms of sulphonal and trional poisoning include stupor, ataxia, constipation, vomiting and gastric pains. Evidences of kidney irritation (albuminuria and nephritis) are also to be seen. In many cases, though not in all, it extends as far as formation of urinary porphyrin. Spectroscopically this behaves very much like haematoporphyrin from haemoglobin though it cannot be identical with that substance. The dark red to brown color of the urine is a frequent but by no means constant symptom of sulphonal and trional poisoning

Detection of Sulphonal and Trional in Urine

Sulphonal occurs in the urine in detectable quantity only after rather large doses, especially following uninterrupted administration of the drug. Elimination of unaltered sulphonal in the urine continues for several days. For this reason it is sometimes possible to find sulphonal even in the organism, when the poisoning terminated fatally several days later. This is a significant fact because death as a rule takes place after the lapse of several days. A sulphonal urine is often colored dark red to garnet-brown due to a content of haematoporphyrin (?). But this decomposition product of blood-pigment appears in urine only succeeding severe poisoning by sulphonal, and even then its occurrence is rare.

To isolate sulphonal from urine, evaporate 1000 cc. or more to about one-tenth its volume, and extract several times with large quantities of ether. Pass the combined ether extracts, after they have settled in a dry flask for several hours, through a dry filter and distil. Evaporate the residue with 20–30 cc. of 10 per cent. sodium hydroxide solution to dryness upon the water-bath. This will remove coloring matter, extracted from urine by ether, but will not affect sulphonal. Extract sulphonal from the alkaline residue with ether. Evaporate the solvent and sulphonal will remain pure and almost colorless. Determine the melting-point of this residue and make the other tests for sulphonal.

Detection of Haematoporphyrin in Urine in Sulphonal Poisoning

Coloring matters have been observed in red, brownish red or cherry-red urines, probably identical with haematoporphyrin. Spectroscopic examination of such urine is made in the following manner. Add sodium hydroxide solution drop by drop to about 0.3 liter of urine, until the reaction is strongly alkaline, and then add a little barium chloride solution. Filter after a while and wash the precipi-

tate well. Extract the precipitate upon the filter with hot alcohol containing a few drops of dilute sulphuric acid. Spectroscopic examination of this filtrate may be made direct with a Browning pocket spectroscope. Acid haematoporphyrin solutions are violet; when more concentrated, they have a cherry-red color and show the characteristic spectrum with two absorption-bands. If the acid, alcoholic solution is saturated with a few drops of ammonium or sodium hydroxide solution, the spectrum of alkaline haematoporphyrin solution with its four absorption-bands appears. Traces of haematoporphyrin very frequently appear in normal urine. See absorption-spectra upon page 328.

TRIONAL

Trional, methyl-ethyl-methane-diethyl-sulphone, $(C_2H_5)(CH_3)$-$C(SO_2C_2H_5)_2$, crystallizes in colorless, shining, inodorous plates melting at 76°. It is soluble in 320 parts of water, the solution having a bitter taste and without action upon litmus paper. In the former property it differs from sulphonal which is tasteless. Trional gives the sulphonal reactions. Trional is completely decomposed in the human organism and danger of cumulative action is much less than in the case of sulphonal. Moreover, haematoporphyrinuria has almost never been observed, even following considerable doses of trional and after uninterrupted use for weeks.

SANTONIN

Santonin occurs to the extent of 2–3 per cent. in the unexpanded flower-heads of Artemisia maritima (Flores Cinae), also falsely called worm-seed. The blossoms after opening contain no santonin. Flores Cinae and santonin find use in medicine as a remedy for round and other intestinal worms.

Santonin, $C_{15}H_{18}O_3$, crystallizes in colorless, inodorous, shining leaflets that have a bitter taste and melt at 170°. Light turns these crystals yellow. Santonin dissolves in 5000 parts of cold and 250 parts of boiling water; in 44 parts of alcohol; in 150 parts of ether; and in 4 parts of chloroform. All these solutions are neutral. Upon evaporation, an alcoholic solution of the yellow modification deposits white santonin. Santonin is the internal anhydride (lactone) of santonic acid, $C_{15}H_{20}O_4$, for caustic alkalies, as well as calcium and barium hydroxides, dissolve santonin forming salts of this acid.

Toxic Action.—In man the principal evidences of poisoning are nervous disturbances of the retina recognized by yellow or violet vision, usually associated with sparkling. Occasionally nervous disturbances of the olfactory and gustatory nerves also appear. Additional manifestations are headache, dizziness,

trembling, convulsions, unconsciousness, vomiting and diarrhoea, icterus and fever. Pronounced slowing of the pulse, dilatation of the pupils, yellow coloration of the conjunctiva and of the urine have been observed. Even 0.06 gram of santonin is said to have caused death. As a rule, however, much larger quantities are tolerated. Elimination of santonin from the body takes place rather slowly. For this reason it acts cumulatively. Consequently poisonings may occur as a result not only of wrong dosage but of too long administration of santonin.

Behavior in the Animal Organism.—Santonin seems to be incompletely absorbed in the organism. Jaffé[1] has administered quite large quantities of santonin to dogs and rabbits. He obtained a new substance, called α-oxysantonin ($C_{15}H_{18}O_4$), from the urine of the dog, amounting to 5–6 per cent. of the santonin administered. He extracted with chloroform considerable quantities of unaltered santonin from the faeces of the dog. Rabbits usually can tolerate being fed with santonin for weeks, and α-oxysantonin is formed only in very small quantity. In the ether extract of rabbit's urine, Jaffé found a second santonin derivative, β-oxysantonin, isomeric with α-oxysantonin, with considerable unaltered santonin. In these experiments only about half the santonin administered was absorbed by the rabbit.

After administration of santonin, a red pigment called santonin-red appears in human urine. Even after medicinal doses santonin urine is red, or becomes at least scarlet-red to purple on addition of potassium or sodium hydroxide solution. Urine containing santonin also becomes carmine-red on addition of calcium hydroxide solution.

Detection of Santonin

Ether, benzene, or better chloroform, extract santonin only from neutral or acid solutions. The organic solvent fails to remove this compound from an alkaline solution, as it is then in the form of a santonate. Santonin is not an alkaloid and forms no precipitates with the general alkaloidal reagents but gives several more or less characteristic color reactions.

1. Alcoholic Potassium Hydroxide Test.—Pure, white santonin, heated with an alcoholic solution of potassium hydroxide, gives a fine carmine-red color that gradually changes to reddish yellow and finally fades entirely. In this test yellow santonin dissolves with a yellowish red color.

2. Sulphuric Acid-Ferric Chloride Test.—If powdered santonin (0.01 gram) is shaken with a cold mixture of 1 cc. of sulphuric acid and 1 cc. of water, no color appears. But if the mixture is then heated almost to boiling with addition of a few drops of ferric chloride solution, it takes on a violet color.

[1] M. Jaffé: Oxy-Santonin and Its Formation in the Animal Body after Administration of Santonin. Zeitschr. f. physiol. Chem. 22 (1896 to 1897), 337.

3. **Furfural-Sulphuric Acid Test.**—Mix 2–3 drops of alcoholic santonin solution with 1–2 drops of 2 per cent. alcoholic furfural solution and 2 cc. of pure concentrated sulphuric acid. Warm this mixture in a small porcelain dish upon the water-bath. A purple-red color appears and changes with continued heating to crimson-red, blue-violet and finally to dark blue (Thäter[1]). Should this delicate santonin test not appear at once, add a few drops more of furfural solution. The play of colors described appears quite distinct with only 0.1 mg. of santonin. In absence of santonin, the furfural-sulphuric acid is colored first pale red, later an off-shade brown.

Only a few alkaloids and glucosides give distinct color reactions with furfural and sulphuric acid. Substances behaving similarly are veratrine, picrotoxin (violet) and piperine (green to blue-green, finally indigo-blue). The colors given by α- and β-naphthol with furfural and sulphuric acid are also characteristic.

4. **Potassium Cyanide Test.**—Heating together a mixture of santonin and powdered potassium cyanide produces a dark red mass dissolving in water, or in solutions of caustic alkalies, with a strong green fluorescence.

Special Examination for Santonin

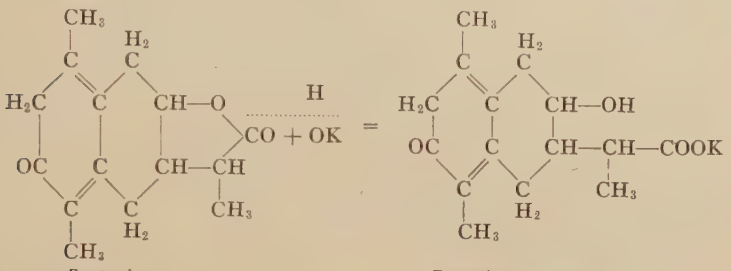
In suspected santonin poisoning, examine vomitus, contents of stomach and intestines, and also, if the poisoning is of very recent occurrence, organs and blood. Urine contains only decomposition products of santonin (see above).

Comminute the material as finely as possible and warm for several hours upon the water-bath with water containing sodium hydroxide, or according to Dragendorff with milk of lime, and then strain. To remove impurities from the liquid thus obtained, first shake with benzene, then acidify with hydrochloric acid, and again extract with benzene, or better with chloroform. Evaporation or distillation of solvent will leave santonin. The final solutions are purer, if the alkaline liquid is first freed from impurities by using about 3 times the volume of alcohol as a precipitant. Collect the precipitate upon paper, remove alcohol from the filtrate by distillation, and thoroughly extract the residual solution with benzene, when alkaline, and with chloroform, when acid. The latter then contains the santonin. Recrystallize crude santonin thus obtained from hot water, using animal charcoal to remove any color.

[1] K. Thaeter: Contributions to Forensic Chemistry. Archiv d. Pharmaz. 235 (1897), 401.

Chemistry of Santonin

Santonin is the internal anhydride (lactone) of santonic acid, $C_{15}H_{20}O_4$. Caustic alkalies, as well as calcium and barium hydroxides, dissolve santonin forming salts of this acid. In this case, as with all lactones, the lactone ring is broken as follows:



Santonin — Potassium santonate

A solution of a santonate, acidified with hydrochloric acid, first gives free santonic acid. To isolate this compound from the mixture, extract at once with ether. Otherwise, the acid loses 1 molecule of water upon standing and passes into its internal anhydride, santonin.

Santonin is also a ketone. As such it forms a hydrazone, $C_{15}H_{18}O_2 = N - NH.C_6H_5$, with phenyl-hydrazine and an oxime, $C_{15}H_{18}O_2 = NOH$, with hydroxylamine.

According to the above structural formula, santonin is a derivative of hexahydro-dimethyl-naphthalene. Fused with potassium hydroxide, santonic acid gives hydrogen, propionic acid, and a naphthalene derivative, namely, dimethyl-β-naphthol.

Examination for Santonin,[1] Sulphonal and Trional

These potent drugs considered in this place do not find a place in the Stas-Otto process on account of their behavior toward cold tartaric acid solution and ether. The following method may be employed for their detection:

Extract the material, neutral or faintly acid with tartaric acid, under a reflux with boiling absolute alcohol. Filter hot and evaporate the filtrate to dryness upon the water-bath. Dissolve the residue in hot water. If the solution is colored, digest it for some time upon the water-bath with bone-black and stir frequently. Filter the hot solution. All of the above substances, if present in considerable quantity, crystallize in part as the solution cools. Thoroughly

[1] Because santonin manifests toward solvents a behavior similar to that of sulphonal and trional and is for that reason obtained by the same method of extraction, it is included in this place in connection with these hypnotics.

extract the filtrate and any crystals several times with chloroform. Pass the chloroform extract through a dry filter. The residue from chloroform may contain santonin, sulphonal and trional.

The chloroform residue may also contain those substances extracted in the Stas-Otto process from the acid solution by ether. Chloroform completely extracts substances such as colchicin, antipyrine, caffeine, acetanilide, phenacetine and salicylic acid. As a rule they are purer from this solvent than from ether. The chloroform residue may also contain the weak base narcotine.

CERTAIN ALKALOIDS OF RARE OCCURRENCE

ACONITINE

Aconitine, $C_{34}H_{47}NO_{11}$, in combination with aconitic acid, occurs with other bases in Aconitum napellus, especially in the plant (0.3 per cent. total alkaloids) and in the tubers (about 0.6 per cent. total alkaloids). The tubers of other aconite species are said also to contain this alkaloid in smaller quantity. Pure aconitine forms colorless, tabular, rhombic crystals dissolving with difficulty in water but readily in alcohol, ether, chloroform and benzene. It is nearly insoluble in petroleum ether. Aqueous solutions of aconitine have a very sharp, persistently burning but not bitter taste. The very bitter taste of aconitine when not entirely pure is due to an admixture of an amorphous alkaloid known as picraconitine.

Chemical Behavior.—Aconitine boiled with water, but more quickly in presence of mineral acid or alcoholic potassium hydroxide solution, undergoes hydrolysis first into acetic acid and the very bitter picraconitine known to be benzoyl-aconine:

$$\underset{\text{Aconitine}}{C_{34}H_{47}NO_{11}} + H_2O = \underset{\text{Acetic Acid}}{C_2H_4O_2} + \underset{\text{Picraconitine}}{C_{32}H_{45}NO_{10}}$$

Upon further hydrolysis the latter yields benzoic acid and aconine:

$$\underset{\text{Picraconitine}}{C_{32}H_{45}NO_{10}} + H_2O = \underset{\text{Benzoic acid}}{C_6H_5.COOH} + \underset{\text{Aconine}}{C_{25}H_{41}NO_9}$$

These reactions show aconitine to be acetyl-benzoyl-aconine. Ordinary commercial preparations of aconitine contain the cleavage-products, picraconitine and aconine, and to their presence may be attributed the bitter, sharp taste as well as the uncertain potency of commercial aconitine.

Reactions.—Even in very dilute solution aconitine is precipitated by such general alkaloidal reagents as phospho-molybdic acid, phos-

pho-tungstic acid, iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, tannic acid, and gold chloride, whereas platinic chloride, mercuric chloride, and picric acid cause precipitation only in more concentrated solutions.

Concentrated sulphuric acid dissolves pure crystallized aconitine without color and concentrated nitric acid behaves in the same way. Careful evaporation of pure aconitine with officinal phosphoric acid produces either no result at all or only a very faint red color.

Physiological Behavior.—Aconitine should be classed with the most powerful poisons. At the outset it stimulates or irritates the terminations of the sensory motor or secretory nerves of the striated muscles, of the heart, and of the central nervous system (brain, spinal cord, emetic center, motor center of the intestines, center of respiration and of dilatation of the pupils). Then they are deadened or paralyzed so that death occurs as a result of paralysis of the heart or lungs. The symptoms of aconitine poisoning are burning in the throat, flow of saliva, vomiting, colic, diarrhoea, insensibility of the tongue, enlargement of the pupil, disturbance or loss of the sense of sight and hearing, marked slowing of the pulse, difficulty in breathing and convulsions. Aconitine is so easily absorbed even after external application that poisoning takes place quickly, in a few days or even hours. Elimination proceeds mainly through the urine which is especially adapted for the isolation of the poison.

Pseudo-Aconitine.—This alkaloid, $C_{36}H_{49}NO_{12}$, is found principally in the tubers of Aconitum ferox. They are said to contain no aconitine. On slow evaporation of an ether solution, or better of a mixture of ether and petroleum ether, pseudo-aconitine crystallizes in transparent needles or granular crystals. On rapid evaporation it is deposited as an amorphous or syrupy mass. Though slightly soluble in water, it is more easily soluble than aconitine in alcohol and ether. Solutions of pseudo-aconitine are dextro-rotatory; those of its salts laevo-rotatory.

Chemical Behavior.—Several hours boiling with water causes hydrolysis of pseudo-aconitine into acetic acid and picro-pseudo-aconitine:

$$\underset{\text{Pseudo-aconitine}}{C_{36}H_{49}NO_{12}} + H_2O = \underset{\text{Acetic acid}}{C_2H_4O_2} + \underset{\text{Picro-pseudo-aconitine}}{C_{34}H_{49}NO_{11}}$$

The latter base is then further hydrolyzed by alcoholic potassium hydroxide solution into veratric acid and pseudo-aconine:

$$\underset{\text{Picro-pseudo-aconitine}}{C_{34}H_{49}NO_{11}} + H_2O = \underset{\text{Veratric acid}}{C_9H_4O_4} + \underset{\text{Pseudo-aconine}}{C_{25}H_{39}NO_8}$$

These reactions show that pseudo-aconitine is acetyl-veratryl-pseudo-aconine.

Reactions.—Pseudo-aconitine resembles aconitine in its behavior toward general alkaloidal reagents. Even in great dilution it is precipitated by potassium mercuric iodide, iodo-potassium iodide, tannic acid, and gold chloride, whereas platinic chloride causes precipitation only in more concentrated solutions. Pure pseudo-aconitine in presence of concentrated sulphuric and phosphoric acids gives no distinctive color reactions. According to Dragendorff, pseudo-aconitine isolated from the organs of animals poisoned by this alkaloid is said to behave like pure aconitine. By Vitali's test pseudo-aconitine behaves like atropine, for a small quantity of this alkaloid evaporated upon the water-bath with a few drops of fuming nitric acid leaves a yellow residue that takes on a fine purple-red color when moistened with alcoholic potassium hydroxide solution (1:10). Absolutely pure crystallized aconitine does not give this test.

In carrying out Jürgens test, Gadamer advises the following procedure. To test for aconitine or pseudo-aconitine in the alkaloidal residue obtained by evaporating the ether extract of the aqueous alkaline solution, dissolve in a drop of water containing acetic acid and add a small crystal of potassium iodide. When the latter has dissolved and the solution has evaporated spontaneously, dissolve the crystallized potassium iodide with a drop of water and quickly remove the solution. The iodide from pure aconitine appears in well-formed, tabular crystals of rhombic shape. Sometimes these crystals are cross-shaped and grow together obliquely, or lie together feather-like. Impure aconitine will not give this reaction but forms only amorphous masses. Pseudo-aconitine gives needle-shaped druses.

Führer's Biological Detection of Aconitine.[1]—To prevent decomposition of the exceedingly labile aconitine, extract the material at moderate heat with alcohol containing only a small quantity of tartaric acid. Remove alcohol from the filtered extract at as low a temperature as possible, best *in vacuo*. Filter the faintly acid, aqueous residue to get rid of fat and extract the filtrate with petroleum ether to remove impurities. Then render the aqueous solution alkaline with sodium carbonate (not with caustic alkali) and at once extract alkaloid with ether. Dissolve the residue left upon evaporating the ether extract in a little water containing acetic acid. Filter the solution, make alkaline with sodium carbonate, and again extract with ether. The residue from this ether extract may be used for Führer's biological test for aconitine. Dissolve the

[1] H. Führer: Toxicological Detection of Aconitine. Arch. f. exper. Path. u. Pharm. 66 (1911), 179.

residue in a little water containing acetic acid, add sodium carbonate to the filtered solution until neutral, dilute to 5 cc., and taste by dipping a strip of purest filter-paper in the solution and applying it for a short time to the tip of the tongue. After 5–10 minutes, if aconitine is present, a burning sensation is noticeable upon the tongue, similar to that often produced by too hot food. This irritation may persist for hours. This test, according to Führer, is characteristic of aconitine. Using the same solution (5 cc.), correspondingly diluted with Ringer's solution isotonic with the frog, Führer also makes a test with isolated frog's heart. By this test 0.05 mg. of aconitine may be detected with certainty. Of the three stages in the action of aconitine on the frog's heart, the second, that of heart convulsions or heart peristalsis, is specific for aconitine. Delphinine, an alkaloid of no forensic significance, alone resembles aconitine in its action upon the heart.

ARECOLINE

Areca-nuts, or betel-nuts, the seeds of Areca Catechu, contain several alkaloids. Areca-palms, originally indigenous to the Sunda islands, are now cultivated to a great extent in the warmer parts of the Indies as well as in the Philippines. Areca-nuts in their habitat are an important article of commerce, for mixed with lime and leaves of the betel pepper they are chewed by the natives. Occasionally they are also used in China and the Indies as a vermifuge. With the exception of choline, the following bases isolated by Jahns[1] are closely related: choline, guvacine ($C_6H_9NO_2$), arecaine ($C_7H_{11}NO_2$, H_2O), arecaidine ($C_7H_{11}NO_2$, H_2O), arecoline ($C_8H_{13}NO_2$). Of these bases arecoline must be viewed as the physiologically active constituent and as the seat of the anthelmintic action of areca-nuts. The yield of arecoline is 0.07, at the highest 0.1 per cent., that of arecaine about 0.1 per cent. (Jahns).

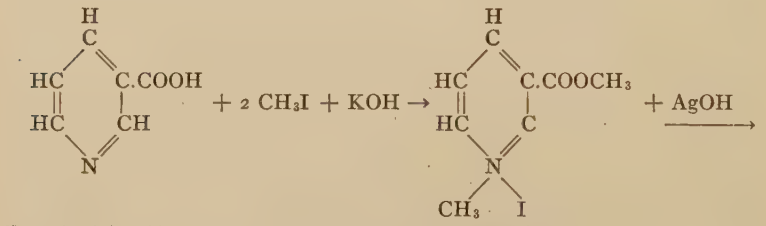
Arecoline is a colorless, oily liquid having a strong alkaline reaction and soluble in all proportions in water, alcohol, ether and chloroform. It boils at about 220° and is also volatile with steam. Most of its crystallizable salts are easily soluble and some are deliquescent. Of the simple salts arecoline hydrobromide ($C_8H_{13}NO_2.HBr$) used in medicine crystallizes best. Solutions of arecoline salts with potassium bismuthous iodide give a garnet-red precipitate consisting of microscopic crystals (delicate reaction), and a white precipitate with phospho-molybdic acid. If the solution is not too dilute, potassium mercuric iodide causes a precipitate of yellow, oily drops that become crystalline after several days, iodo-potassium iodide brown drops, and picric acid a resinous precipitate later changing to needles. Platinic chloride, mercuric chloride and tannic acid cause no precipitation. Arecoline gives no characteristic color reactions.

Physiological Action and Use.—Arecoline differs from the other areca-alkaloids in being strongly active. Its action is very much like that of pilocarpine. But its action upon the pupil of the eye, causing contraction, is even stronger than that of the latter and in this respect is closely related to the action of muscarine.

[1] E. Jahns: The Alkaloids of Areca Nuts. Berichte d. Deutsch. chem. Ges. 21 (1888), 3404; 23 (1890), 2972; 24 (1891), 2615.

Introduced into a cat's eye it may cause the pupil to disappear almost completely (Kobert). In its anthelmintic action it resembles pelletierine. Injected directly into the blood of dogs in almost lethal doses, it gives rise to convulsive attacks followed by paralysis of the central nervous system. In addition it increases mucous, salivary and sweat secretion. Arecoline in part is said to be eliminated undecomposed in the urine. The lethal dose of arecoline for a rabbit is 10 mg. On the other hand, rather small dogs have survived doses of 50–75 mg. of the base. Arecoline resembles muscarine in its action upon the heart. Taken internally it causes ejection of intestinal parasites, especially tapeworm. Arecoline hydrobromide in doses of 0.004–0.006 gram is used for this purpose. A 1 per cent. solution of this salt serves as a myotic, that is, an agent to cause contraction of the pupil in ophthalmology. In subcutaneous doses of 0.03–0.06 gram it is used in veterinary medicine as a remedy for colic in horses.

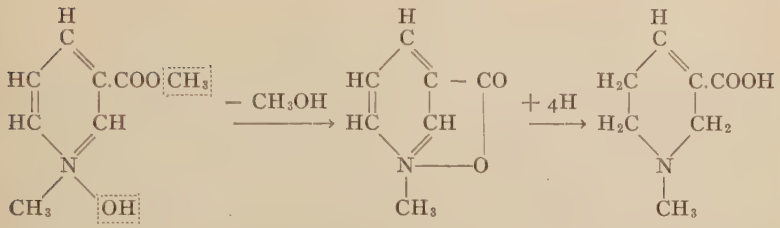
Constitution and Synthesis of Arecoline.—Heated with strong hydrochloric acid to about 150°, arecoline is decomposed into methyl chloride and arecaidine. On the other hand, arecaidine which has weak acid properties may be converted into arecoline by methylation, that is, by passing dry hydrochloric acid gas into a suspension of arecaidine in methyl alcohol. Arecaidine has been synthesized. This was accomplished by starting from nicotinic acid, or β-pyridine carboxylic acid, and passing through trigonelline[1] which also has been synthesized. Nicotinic acid is evaporated with potassium hydroxide (1 mol.) and the dry residue heated to 150° with excess of methyl iodide. The iodomethylate of the methyl ester of nicotinic acid thus formed is digested with moist silver oxide. Careful evaporation of the filtrate leaves trigonelline, or the methyl-betaine of nicotinic acid, as colorless needles readily soluble in water and less so in alcohol. Trigonelline, or the iodomethylate of the methyl ester of nicotinic acid, is reduced when heated for some time with tin and hydrochloric acid. The product of reduction is then freed from tin by hydrogen sulphide. The resulting solution treated with silver oxide and then freed from silver by hydrogen sulphide yields methyl-hexahydro-nicotinic acid. This compound can be extracted with chloroform and is identical with dihydro-arecaidine. A part insoluble in chloroform and formed at the same time, after treatment with absolute alcohol, leaves undissolved methyl-tetrahydro-nicotinic acid identical with arecaidine:



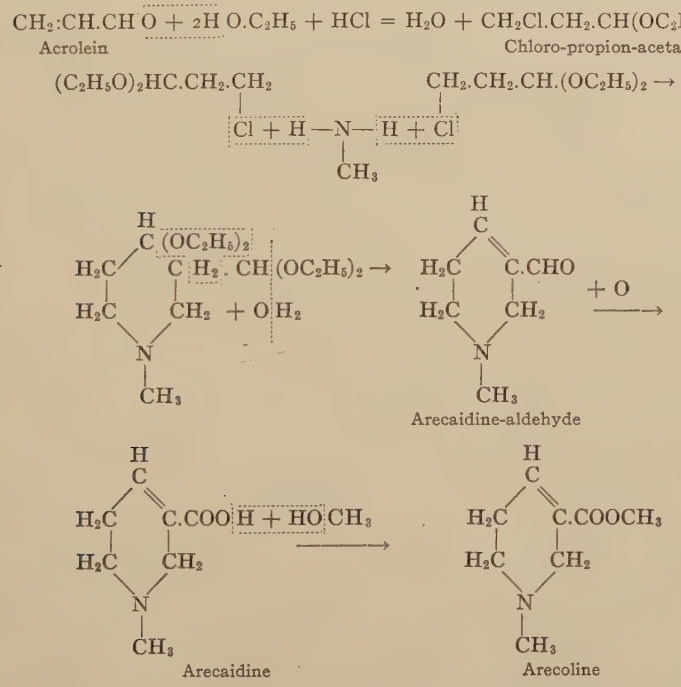
Nicotinic acid

Iodo-methylate of methyl ester of nicotinic acid

[1] Trigonelline occurs to the extent of about 0.15 per cent. in the seeds of Trigonella foenum graecum and also in peas, hemp-seed and oats.



According to a synthesis of arecaidine discovered by Wohl and Johnson,[1] acrolein with alcohol is converted by means of hydrochloric acid gas into chloro-propion-acetal. The latter is heated to 125° with methylamine dissolved in benzene. Treatment of the base thus formed with fuming hydrochloric acid at 0° causes ring formation. The product is the hydrochloride of arecaidine-aldehyde which is converted by oxidation into arecaidine. Dry hydrochloric acid gas passed into a suspension of the latter in methyl alcohol converts it into arecoline:



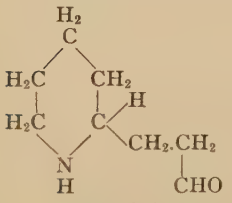
[1] A. Wohl and A. Johnson: Arecaidine and Arecoline. Berichte d. Deutsch. chem. Ges. 40 (1907), 4712.

Detection of Arecoline

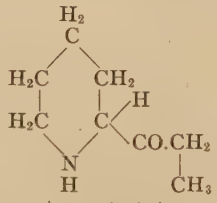
Volatility of free arecoline base with steam may be used to effect its separation. Its action in causing contraction of the pupil of the eye may be employed for its recognition, since it has no characteristic chemical reactions. To carry out this test, dilute the material with water, render alkaline with sodium hydroxide solution and distil. Exactly neutralize the distillate with dilute hydrochloric acid, evaporate to a few drops, and introduce the solution into a dog's eye, or better a cat's eye. Contraction of the pupil indicates presence of arecoline. An alternative procedure is to extract the distillate with ether, evaporate the ether extract, neutralize the residue with very dilute hydrochloric acid, and use this solution for tests upon the eye.

Alkaloids of Pomegranate Bark

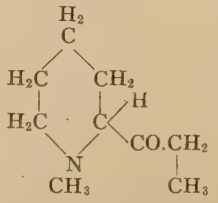
The bark of the root and trunk of the pomegranate tree (Punica granatum) contains several alkaloids. Tanret (1878) was the first to isolate from this bark four volatile bases to which the names, pelletierine, isopelletierine, pseudo-pelletierine and methyl-pelletierine were given. As the fifth member of this group, Piccini added iso-methyl-pelletierine. In his description of the bases isolated and in the analyses of their salts Tanret characterized them as chemical individuals and gave them their true composition. He did not undertake to determine their chemical structure. Pseudo-pelletierine, $C_8H_{15}NO$, which crystallizes well and occurs in largest quantity in pomegranate bark, was recognized by Ciamician and Silber as a higher homologue of tropinone. This base was very useful to R. Willstätter in the synthesis of cyclo-octane derivatives. As a result of numerous recent investigations by Kurt Hess[1] and his collaborators, the constitution of the alkaloids in pomegranate bark is accurately known. None of these bases is optically active. Hess was unable to find the active alkaloids described by Tanret and Piccini. It is probable that impurities were responsible for the mistaken conclusion that optically active bases were present. The bases of known constitution occurring in the bark of Punica granatum are the following:



Pelletierine

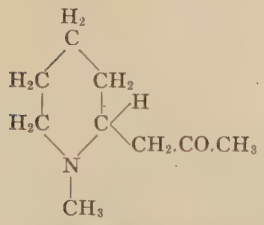


Isopelletierine

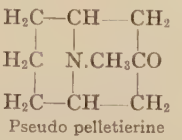


Methyl isopelletierine

[1] K. Hess: Alkaloids of the Pomegranate Tree. I. Pelletierine. Berichte d. Deutsch. chem. Ges. 50 (1917), 368; II. Methyl-pelletierine of Tanret and Isomethyl-pelletierine of Piccini. Ibid. 50 (1917), 380; III. Constitution of Pelletierine. Ibid. 50 (1917), 1192; IV. Method of Separation for the Purification of Pelletierine Alkaloids. Ibid. 50 (1917), 1386; V. Cleavage of Pelletierine and Methyl-isopelletierine into Their Optical Isomers: Explanation of Tanret's Bases. Ibid. 51 (1918), 741; VI. Relations of Methyl-isopelletierine, etc. Ibid. 52 (1919), 964; VII. Natural Occurrence of isopelletierine. Ibid. 52 (1919), 1005.



1-(α-N-Methyl-piperidyl)-propane-2-one



Pseudo pelletierine

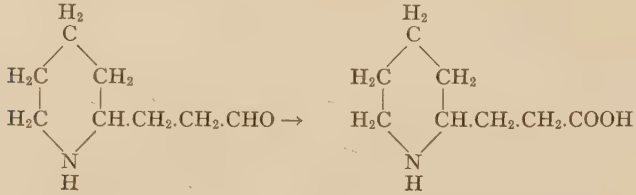
Isolation of Alkaloids by Hess and Eichel (IV Paper)

To get total alkaloids from the bark into solution, add milk of lime to the finely powdered bark and extract with chloroform. Then extract the alkaloids from chloroform solution with dilute sulphuric acid. Add excess of sodium hydroxide solution to this acid aqueous solution of alkaloidal sulphates and extract with ether in a Kempf apparatus. Dry the ether solution with potassium carbonate, filter to remove particles of resinous matter, and fraction *in vacuo*. The first portion of distillate contains lower boiling fractions. The fraction from about 100–120° is a mixture of pelletierine and methyl-isopelletierine. At 145° pseudo-pelletierine begins to distil and solidifies in the receiver to a crystalline mass. Distillation at this point is stopped. The residue in the distilling flask is dissolved in petroleum ether and the solution placed in ice. Pure pseudo-pelletierine crystallizes. To separate pelletierine and methyl-isopelletierine, treat the fraction containing these alkaloids with ethyl-chloro-formate, $Cl.CO.OC_2H_5$. The secondary base, pelletierine, forms a urethane, whereas the tertiary base, methyl-isopelletierine, does not. If the mixture is now fractionally distilled, methyl-isopelletierine boils at 105–106° (15 mm.) and pelletierine urethane at 169–170°. The latter heated with fuming hydrochloric acid at 125–135° gives pure pelletierine through elimination of ethyl chloride and carbon dioxide. On the other hand, if crude pelletierine urethane is saponified with alcoholic sodium hydroxide, the liberated base is completely resinified with formation of non-volatile, resinous products. But isopelletierine urethane present in small quantity is readily saponified and this alkaloid can then be distilled.

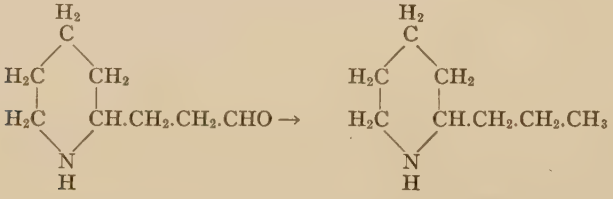
The picrates of the alkaloids in pomegranate bark are especially characteristic, for they are well-crystallized and have definite melting-points. Pelletierine picrate melts at 150°; isopelletierine picrate at 154°; methyl-isopelletierine picrate at 155°; and pseudo-pelletierine picrate at 252-253°.

Pelletierine, $C_8H_{15}NO$, is a colorless oil absorbing oxygen from air with avidity and turning brown from resinification. It has an alkaline reaction and a characteristic spicy aromatic odor. It is soluble in 20 parts of water and in ether, alcohol and chloroform in all proportions. Pelletierine sulphate, $(C_8H_{15}NO)_2$-H_2SO_4, a white crystalline non-hygroscopic compound, and pelletierine tannate are used in medicine as a remedy for tapeworm. The nitrogen of pelletierine, according to Hess, is secondary, since it forms an N-acetyl- and an N-benzoyl-derivative that crystallize especially well. Presence of an aldehyde group has also been shown, since pelletierine forms an oxime. Cleavage of water from the latter by treatment with phosphorus pentachloride gives a nitrile that may be

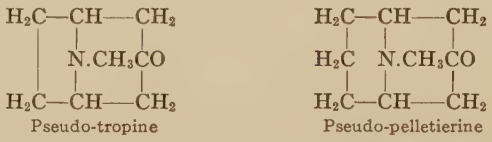
converted by saponification into p-hexahydro-2-pyridyl-propionic acid (pelletieric acid) obtained synthetically:



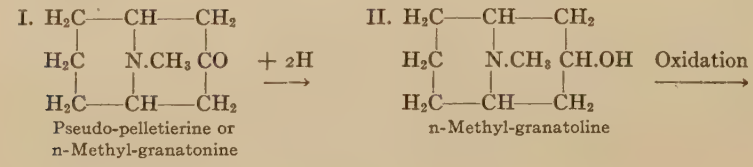
Pelletierine is evidently an oxidation product of coniine. Hess and Eichel in fact succeeded in converting pelletierine into coniine. Pelletierine is converted quantitatively by hydrazine hydrate into a hydrazone. The latter heated with sodium ethylate is converted nearly quantitatively into racemic coniine:

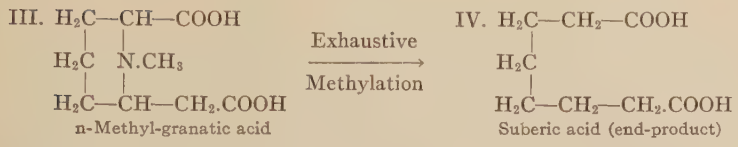


Pseudo-pelletierine, $C_9H_{15}NO + 2H_2O$, occurs mainly in the bark of the trunk of Punica granatum and in smaller quantity in the root. It forms colorless crystals that melt at 48° and readily dissolve in water, alcohol and ether, As a rule its salts crystallize well. It is both a tertiary base and a ketone, the next higher homologue of pseudo-tropine:



Ciamician and Silber found the constitution of pseudo-pelletierine (I) to be that of a ketone, n-methyl-granatonine. As such it is converted by the action of nascent hydrogen, that is, by sodium amalgam, or sodium and alcohol, into the corresponding secondary alcohol, n-methyl-granatoline (II). The latter is oxidized by chromic acid to n-methyl-granatic acid (III) from which the nitrogen atom may be removed by exhaustive methylation giving as the final product suberic acid (IV):





From 100 kilograms of the bark of Punica Granatum C. Hess obtained:

50 grams Pelletierine hydrobromide = 32 grams Pelletierine base
32 grams Pelletierine urethane = 20.5 grams Pelletierine base
total 52.5 grams,

179 grams Pseudo-pelletierine,
22 grams Methyl-isopelletierine,
about 1.5 grams Isopelletierine,
about 1 gram 1-(α-N-methyl-piperidyl)-propane-2-one.

So about 0.25 per cent. of alkaloids may be obtained in pure condition. The mixture of crude alkaloids amounts to about 0.4 per cent. The alkaloid that preponderates in the plant is pseudo-pelletierine. In view of the fact that a considerable quantity of resinous material remains in the distilling-flask from the distillation of the crude alkaloids, and since most of this product very likely comes from the highly sensitive pelletierine, there is reason to believe that the latter is the chief alkaloid of the pomegranate tree. Isopelletierine and 1-(α-N-methyl-piperidyl)-propane-2-one occur only in insignificant quantities. Hess regards these two alkaloids as intermediate stages in the formation of methyl-isopelletierine and pseudo-pelletierine and consequently they disappear soon after formation.

Therapeutic Use and Toxic Action of Pelletierine

Of the pomegranate alkaloids, pelletierine is the most potent and the one used most frequently as a remedy for tapeworm. For rabbits per kilogram of body-weight the lethal dose is 12 mg. injected subcutaneously. The effects of the poison upon man, and upon warm-blooded animals in general, are muscular rigidity like that caused by veratrine and convulsions. Pelletierine may also produce in man diarrhoea with vomiting, colic, slow pulse, collapse, profuse sweat, headache, muscular rigidity, but particularly disturbances of vision. Even medicinal doses of 0.4–0.5 gram of pelletierine sulphate or tannate given for tapeworm may give rise to slight temporary effects of this nature. Rigor and fever, as quite troublesome secondary effects resulting from use of a decoction of pomegranate bark, have also been repeatedly observed. Pelletierine as well as isopelletierine has a very powerful toxic action upon tapeworm. Usually 0.3–0.4 gram of pelletierine sulphate or tannate is sufficient to effect a cure without producing at the same time very severe toxic effects.

Detection

The free alkaloids of pomegranate bark are soluble in ether and chloroform and consequently in the Stas-Otto process they appear in the ether extract of the alkaline aqueous solution. The free bases are volatile with steam. Their odor is characteristic and recalls coniine. Of the general alkaloidal reagents

picric acid would seem to be the best to use as a precipitant, for the picrates of pelletierine and pseudo-pelletierine crystallize well and also have definite melting-points (see above). At the present time the alkaloids of pomegranate bark are without reactions of identification. Concentrated sulphuric acid and the reagents of Erdmann, Froehde and Marquis fail to give characteristic colors.

Alkaloids of Ipecacuanha

True ipecacuanha root from Uragoga ipecacuanha certainly contains three alkaloids and, according to Hesse,[1] five. Emetine and cephaeline are present in largest quantity and give rise to the most notable physiological properties of this root. In a total alkaloidal content of 2.7 per cent., Carr and Pyman[2] found in ipecacuanha 1.35 per cent. of emetine, 0.25 per cent. of cephaeline and a smaller quantity of psychotrine.

The composition of these alkaloids is as follows:

Emetine { Hesse: $C_{30}H_{40}N_2O_4 = C_{26}H_{28}(OCH_3)_4N_2$,
Emetine { Carr and Pyman: $C_{29}H_{38}N_2O_4 = C_{25}H_{27}(OCH_3)_4(NH)(N)$.

Cephaeline: $C_{28}H_{38}N_2O_4 = C_{25}H_{27}(OH)(OCH_3)_3(NH)(N)$.

Psychotrine: $C_{28}H_{36}N_2O_4 = C_{25}H_{26}(OH)(OCH_3)_3(N)(N)$.

Carr and Pyman and also Karrer[3] assign to emetine the empirical formula, $C_{29}H_{40}N_2O_4$. Cephaeline differs in composition from emetine in having one less methylene (CH_2) group. Emetine has been definitely established as a secondary-tertiary base.

Paul and Cownley have also obtained emetine, cephaeline and psychotrine from ipecacuanha. Separation of psychotrine depends upon the fact that unlike emetine and cephaeline it is almost insoluble in ether. Consequently when an aqueous extract of the drug is made alkaline and extracted with ether, psychotrine is not removed. But it may be extracted with chloroform. Moreover cephaeline being a phenol is soluble in sodium hydroxide solution and so cannot be extracted by ether from a solution to which this alkali has been added. On the contrary, emetine under these conditions is dissolved by ether. If the sodium hydroxide solution is acidified with hydrochloric acid and again made alkaline with ammonia, ether will then extract cephaeline. After two or three trials of this method depending upon this difference in behavior toward sodium hydroxide, it is possible to make an approximately quantitative separation of the two alkaloids, since emetine too is somewhat soluble in sodium hydroxide (O. Keller). By their method, Paul and Cownley found in Rio root 1.45 per cent. of emetine, 0.52 per cent. of cephaeline, and about 0.04 per cent. of psychotrine; in Carthagena root 0.89 per cent. of emetine, 1.25 per cent. of cephaeline, and 0.06 per cent. of psychotrine.

[1] O. Hesse: Contribution to the Knowledge of the Alkaloids of True Ipecacuanha. Annalen der Chemie 405 (1914), 1.

[2] Carr and Pyman: Ipecacuanha Alkaloids. Journ. Chem. Soc. 105 (1914), 1591.

[3] P. Karrer: Ipecacuanha Alkaloids. Berichte der Deutsch. chem. Ges. 49 (1916), 2057.

Hesse's Isolation of Ipecacuanha Alkaloids.—Mix finely ground ipecacuanha with sufficient cold sodium carbonate solution (1 part of anhydrous sodium carbonate: 3 parts of water) so that it is well moistened but does not gather into balls. Then extract at 60–70° with about 8 times its weight of benzene-benzine (1:5). Remove the free alkaloidal bases from the latter solution by means of 0.1 n-sulphuric acid. Add ammonia in slight excess to this solution and take up the free bases in ether. As a rule six extractions are sufficient to remove emetine and cephaeline completely from the root with hot benzene-benzine and also with ether from the alkaloidal sulphate solution rendered alkaline with ammonia. Distil the ether solution to about one-third of its original volume and extract repeatedly with 0.2 n-sodium hydroxide solution to effect the separation of cephaeline from emetine. Cephaeline is extracted completely and only traces of emetine. Continue extraction with 0.2 n-sodium hydroxide solution until the latter tested with ammonium chloride shows no turbidity. Dilute the alkaline cephaeline solution with an equal volume of water, add hydrochloric acid in slight excess, and saturate with ammonia. Free cephaeline is precipitated, taken up in ether, and obtained in long needles by allowing this solution to evaporate.

Mix the ether solution of emetine, from which cephaeline has been removed, with an ether solution of oxalic acid as long as this produces a white precipitate usually flocculent. To observe the effect of this reagent better, add it at intervals of one minute. If the oxalate precipitate is flocculent, addition of a few drops of water will make it more compact. Decant the ether from the oxalate precipitate and dissolve the latter in warm water. If 500 grams of ipecacuanha are taken, 50–60 cc. of warm water will dissolve the oxalate precipitate. Add to this solution saturated aqueous sodium bromide solution until it produces a milky turbidity. Emetine is precipitated as hydrobromide. This dissolves with difficulty in cold water and is filtered off after 48 hours. Addition of ammonia to the solution thus obtained produces a white, flocculent precipitate consisting in reality of the alkaloid called ipecamine by Hesse. To remove attendant impurities from precipitated emetine hydrobromide, crystallize it several times from hot water adding at first a little sodium bromide. Finally the hot solution of hydrobromide is cooled and treated with a slight excess of ammonia. Emetine appears as a white flocculent precipitate that soon gathers into a dense mass. Protect this precipitate well from light, otherwise it will turn yellowish or even yellow. Emetine well washed with cold water and air-dried retains about 1 per cent. of moisture easily removed in desiccator over sulphuric acid.

Emetine

Emetine is precipitated from solutions of its salts by ammonia and potassium or sodium hydroxide solution in white flocks that form a white powder when air-dried, or a white mass that is easily reduced to powder. As yet emetine has not been obtained in crystalline form. Emetine is nearly insoluble in cold water, dissolves but slightly in water containing ammonia, and potassium or sodium hydroxide, and is easily extracted from these solutions by ether or chloroform. It is readily soluble in alcohol, ether, acetone, chloroform, and hot benzene. Emetine solutions exposed to sunlight quickly turn yellow. Solutions in dilute acids are also gradually colored by light. An alcohol solution of emetine is

laevo-rotatory. Emetine salts have the following characteristics. Some of these compounds crystallize well, 1 molecule of alkaloid combining with 2 molecules of monobasic acid or with 1 molecule of dibasic acid. Emetine therefore is a di-acid base. Emetine hydrobromide, $C_{30}H_{40}N_2O_5.2HBr.4H_2O$, crystallizes in white needles that dissolve very easily in hot water but only slightly in cold water and alcohol. In desiccator it completely loses its water of crystallization. It is dextro-rotatory.

Detection of Emetine.—Concentrated sulphuric acid dissolves pure emetine without color. If, however, sulphuric acid containing molybdic acid, that is, Froehde's reagent, is added to this solution, it develops an intense emerald-green color within a few minutes. This color persists for days, if the solution is kept in desiccator protected from moist air.

Cephaeline

Cephaeline crystallizes from ether in long, delicate, white needles that easily dissolve in ether, alcohol, acetone, chloroform, methyl alcohol, and hot benzene when freshly precipitated. Mixed with 0.5 n-potassium hydroxide solution in equal molecular proportions, cephaeline only partly dissolves, since the potassium salt is formed as the crystals in part swell up. Gentle warming at once causes solution from which ether will not extract cephaeline. But ether will dissolve all the cephaeline following addition of an equivalent quantity of ammonium chloride. Added to solutions of the alkaloid in dilute acids, potassium or sodium hydroxide solutions produce white, flocculent precipitates that redissolve in excess of precipitant. This behavior establishes cephaeline as a phenolic base. Cephaeline has a faintly bitter taste and in alcoholic solution reacts strongly alkaline toward litmus. Its alcoholic solution is laevo-rotatory: $[\alpha]_D^{15} = -21.2°$. Cephaeline completely neutralizes acids. One molecule of alkaloid combines with 2 molecules of monobasic acid or with 1 molecule of dibasic acid.

Detection of Cephaeline.—A little ferric chloride solution added to an alcoholic cephaeline solution produces a red-brown color that soon changes to blue-green as cephaeline hydrochloride forms. Concentrated sulphuric acid dissolves pure cephaeline without color. Addition of a solution of pure ammonium molybdate in concentrated suphuric acid (sp. gr. 1.84) at once colors the cephaeline solution brown-red soon changing to blue, finally to green, and fading out in a few hours.

Calcium Hypochlorite Test.—Calcium hypochlorite solution develops color when added to solutions of ipecacuanha alkaloids in dilute hydrochloric acid, that given by emetine being yellow and by cephaeline red-yellow. In this test Keller uses glacial acetic acid as the solvent of the two alkaloids.

Psychotrine

When the ipecacuanha alkaloids are extracted by amyl alcohol and removed from this solvent with dilute sulphuric acid, psychotrine, according to Paul and Cownley,[1] remains in this acid solution after it has been saturated with ammonia and shaken with ether which extracts emetine and cephaeline. This is also the case, as mentioned above, when benzene-benzine mixture is used in Hesse's method in place of amyl alcohol. Traces of psychotrine of course pass into the ether. The aqueous ammonia solution is extracted with small quantities of chloroform as long as it continues to show blue fluorescence. The chloroform solution is then shaken with dilute sulphuric acid and the latter solution saturated with a little ammonia. This produces a yellowish, amorphous precipitate that soon changes into small crystals. Finally, psychotrine is recrystallized from a little hot alcohol or acetone mixed with four times the volume of water. Psychotrine, $C_{28}H_{36}N_2O_4 = C_{25}H_{26}(OCH_3)_3(OH)(N)(N)$, is obtained in splendid crystals having the lustre of diamonds. But they effloresce losing 4 molecules of crystal water and at the same time their lustre. Viewed in one direction these crystals appear colorless or yellowish; in other directions red or steel-blue. Anhydrous psychotrine dried at 100° melts at 122°. Crystalline psychotrine is practically insoluble in ether but when first precipitated freely dissolves in this solvent soon separating again except for traces. It dissolves freely in alcohol, acetone and chloroform. Upon addition of water, psychotrine separates from alcohol or acetone in splendid crystals; $[\alpha]_D^{15} = +69.3°$. The ammonia solution of psychotrine, like solutions in water, alcohol or acetone, has a strong blue fluorescence. Psychotrine is precipitated from solutions of its salts by sodium or potassium hydroxide solution in white flocks easily soluble with heat in excess of alkali. It has a very bitter taste and easily causes nausea. In alcoholic solution psychotrine completely neutralizes hydrochloric and hydrobromic acids. A little ferric chloride added to an aqueous solution of the hydrochloride produces a blue color. In this respect it behaves like the phenolic base morphine. Upon reduction in hot alcohol with sodium, psychotrine takes up 2 atoms of hydrogen giving cephaeline and isocephaeline (Carr and Pyman).

Reactions and Detection of Emetine and Cephaeline[2]

Concentrated nitric acid dissolves emetine and cephaeline with orange-yellow color.

Glacial acetic acid in presence of a little calcium hypochlorite solution dissolves emetine yellow and cephaeline red-yellow.

Concentrated sulphuric acid with addition of a few drops of ferric chloride solution dissolves emetine and cephaeline yellow with a tinge of green.

[1] R. H. Paul and A. J. Cownley: Chemistry of Ipecacuanha. Pharmac. Journ. and Transact. (3) 25 (1894/95,) 111 and 690.

[2] A. H. Allen and G. E. Scott-Smith: Certain Reactions of the Alkaloids of Ipecacuanha. Pharmaz. Journ. (4) 15 (1902), 552.

Formaldehyde-sulphuric acid dissolves emetine with green color that turns yellow after 4 hours; cephaeline is deep yellow at once and the color is stable.

Froehde's reagent dissolves emetine with a splendid emerald-green color that presists for more than 24 hours. Cephaeline gives a deep red-violet becoming brown, olive-green, green, and later yellow. The same reagent containing 1 drop of fuming hydrochloric acid dissolves emetine with green color having a faint yellow tint; cephaeline intense blue-green. Emetine allowed to stand with Froehde's reagent in a shallow dish gives a splendid emerald-green slowly turning dark blue from the margin, cephaeline violet quickly turning dark blue from the margin.

The behavior of these alkaloids with Froehde's reagent, according to Allen-Scott-Smith, is especially characteristic when carried out in the following manner: emetine gives a dirty green color changing to light grass-green on addition of hydrochloric acid; cephaeline a purple color changed by hydrochloric acid to Prussian blue; and psychotrine a dark purple changed by hydrochloric acid to pale green. The mixed alkaloids of ipecacuanha also give with great distinctness the Prussian blue reaction of cephaeline upon addition of hydrochloric acid and in this way they may be distinguished from the opium alkaloids.[1]

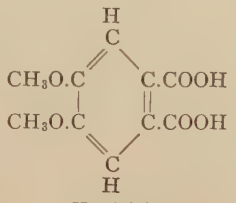
Reduction Test.—The alkaloids of ipecacuanha as derivatives of catechol exert a reducing action, for example, upon a mixture of potassium ferricyanide and ferric chloride with formation of a blue color. A precipitate of Prussian blue is obtained when cephaeline or psychotrine is used. Obviously this test is not characteristic of the alkaloids of ipecacuanha, for morphine behaves in the same way.

Constitution of Emetine and Cephaeline

Methoxyl-groups in emetine and cephaeline are easily determined by Zeisel's method. This test shows that emetine contains four and cephaeline only three such groups. Karrer states that these demethylated compounds may be easily isolated and he has shown that the new base thus obtained from cephaeline is identical in every respect with the base obtained in the same way from emetine and called emetoline. With a drop of ferric chloride solution the liberated compound purified from alcohol gives a deep green color changing to violet upon addition of ammonia. It also reduces ammoniacal silver nitrate solution even in the cold. In other words, it manifests all the catechol reactions. Since emetine has four and cephaeline three methoxyl-groups, emetine is simply

[1] O. Keller: Researches upon the Alkaloids of Ipecacuanha. Archiv d. Pharmazie 149 (1911), 51.

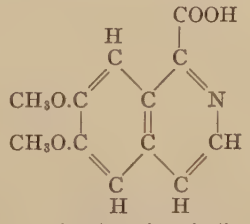
the methyl ether of cephaeline, that is, in cephaeline three of the four hydroxyl-groups are methylated, whereas in emetine all four are methylated! Emetine therefore bears the same relation to cephaeline that codeine does to morphine. A phenolic hydroxyl-group is present in the cephaeline molecule. This much may be said with regard to the relative positions of these four groups, that at least two are ortho to each other, for emetine gives the reactions of catechol. This view is also in accord with the formation of m-hemipinic acid by oxidizing emetine (Windaus).



m-Hemipinic acid

In accordance with the above results methylation of cephaeline should give emetine. Karrer has shown that this is the case. Gentle warming in a tube for several hours of a methyl alcohol solution of cephaeline, containing 2 atoms of sodium with 1 molecule of dimethyl sulphate, gives a good yield of an alkali-insoluble compound. Pure emetine may be obtained from this product by means of the beautifully crystalline hydriodide. Emetine prepared in this way is entirely identical in chemical and biological properties with emetine as it occurs in nature. Cephaeline may be methylated better and more simply by means of nascent diazo-methane.

On the basis of the researches of Keller, Hesse, Carr and Pyman, the nitrogen atoms in emetine and also in cephaeline have been shown in one case to be tertiary and in the other secondary. Most likely one nitrogen atom at least is in an isoquinoline ring, for Carr and Pyman found among the oxidation products of emetine a very small quantity to be sure of 6,7-dimethoxy-isoquinoline-1-carboxylic acid. One of the two nitrogen atoms of emetine belongs to two rings at the same time (Karrer).



6, 7-Dimethoxy-isoquinoline-1-carboxylic acid

Physiological Action and Therapeutic Use

Emetine is an excellent remedy that is specific for amoebic dysentery. Emetine hydrochloride or hydrobromide has proved an excellent expectorant in the

treatment of bronchitis and other catarrhal affections of the respiratory tract. Introduced into the stomach in rather large doses of 0.015 gram, the hydrochloride to be sure causes emesis after about three-quarters of an hour, but not in doses of only 0.005 gram. Cephaeline hydrochloride causes severe vomiting even in doses of only 0.005 gram. According to Lewin, cephaeline is the best emetic, emetine a powerful expectorant, whereas psychotrine is said to have no specific action. The use of emetine salts as a remedy for amoebic dysentery is of very great importance, for this malady is promptly cured by subcutaneous injection of emetine hydrochloride, or hydrobromide, without irritation of the mucous membranes at the same time (Rogers). The total daily dose is 0.03–0.04 gram. Emetine is effective because it kills Entamoeba dysenterica, the cause of this dysentery, and thus renders it innocuous. On the contrary, emetine is without effect upon Bacillus dysentericus, the cause of bacilli-dysentery. Subcutaneous injection of emetine salts speedily cures chronic dysentery arising from amoebic dysentery. Emetine, according to Pick and Wasicky,[1] causes paralysis of the smooth muscles of the blood-vessels of the frog and mammals, also of the muscles of the digestive tract, the bronchial muscles, and finally the protozoa. This paralyzing action does not make the nerves but only the muscles the point of attack. Paralysis caused by emetine is counteracted by barium chloride and vice versa the effect of barium is removed by emetine.

YOHIMBINE

Yohimbine, $C_{22}H_{30}N_2O_4$, together with other alkaloids occurs in Yohimbe bark obtained from West Africa. This alkaloid was first obtained in a state of purity by L. Spiegel in 1896. It appears in white needles melting at 234° and turning yellowish upon exposure to light. It is almost insoluble in water but freely soluble in alcohol, ether and chloroform. Yohimbine at 120° forms the anhydride, $C_{22}H_{28}N_2O_3$. The hydrochloride of this latter body, $C_{22}H_{28}N_2O_3.HCl$, used in medicine, is formed when the base is converted into its hydrochloride. Yohimbine is a dextro-rotatory, tertiary base containing one methoxyl and one hydroxyl-group. If the alkaloid is allowed to stand for 24 hours in contact with potassium hydroxide solution, it loses methyl alcohol and is converted into the potassium salt of yohimbic acid. On the other hand, a mixture of free yohimbic acid and methyl alcohol saturated with dry hydrochloric acid gas yields a precipitate of yohimbine hydrochloride. Upon the basis of this behavior yohimbine is to be regarded as the methyl ester of yohimbic acid.

Physiologically yohimbine bears a certain resemblance to cocaine, for like the latter alkaloid it acts as a local anaesthetic and additionally causes enlargement of the blood-vessels. It enlarges the blood-vessels of the skin, kidneys and intestines and increases the flow of blood to the external genitals. After internal administration, Fr. Müller observed an action upon respiration, the circulation of the blood, and the genital reflexes. Even in small doses it causes increased irritability of the respiratory centre.

If the general procedure for detecting organic poisons by the Stas-Otto process is followed, yohimbine will be extracted by ether or chloroform from the aqueous solution made alkaline with sodium hydroxide solution.

[1] E. Pick and R. Wasicky: Archiv f. experim. Pathol. und Pharmak. 80 (1916), 147.

Detection

Yohimbine reduces ammoniacal silver nitrate solution. As a rule the precipitates given by this base with general alkaloidal reagents are heavy. Concentrated sulphuric acid dissolves yohimbine without color. A crystal of potassium dichromate introduced into this solution produces violet-red streaks. With the reagents of Froehde, Mecke and Mandelin a deep blue color appears at once. Beginning at the margin this color gradually changes to green. A drop of Melzer's reagent (1 gram of benzaldehyde dissolved in 4 grams of absolute alcohol), added to yohimbine on a watch-glass and followed by 1–2 drops of concentrated sulphuric acid, produces at first a dark brown color. Beginning at the margin this color gradually changes to cherry-red and violet, the entire liquid finally becoming violet.

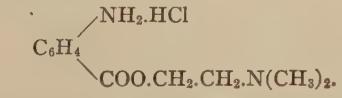
COCAINE SUBSTITUTES

The compounds to be considered in this connection are: Novocaine, Orthoform, Stovaine, Tutocaine, Psicaine, Tropacocaine, together with Adrenaline.

Within recent years various artificial cocaine substitutes have been recommended as local anaesthetics. Belonging to this class are such compounds as acoine, alipine, anaesthesine, eucaine B, holocaine, nirvanine, novocaine, orthoform, stovaine, tutocaine, psicaine, and many others. All these synthetic cocaine substitutes like cocaine itself are bases that can be extracted by ether or chloroform from an alkaline aqueous solution. Consequently if the Stas-Otto process is followed, these bases will be found in the ether extract where the true alkaloids appear. Most of the general alkaloidal reagents produce precipitates in solutions of their salts. Like cocaine the free bases as well as their salts cause temporary insensibility when applied to the tongue.

NOVOCAINE

Novocaine is the hydrochloride of the dimethyl-amino-ethyl ester of para-amino-benzoic acid:



This compound crystallizes in colorless, inodorous needles that have a faintly bitter taste. It dissolves in 1 part of water, 30 parts of alcohol, and with great difficulty in ether. Its aqueous solution has a neutral reaction. Alkalies, alkaline carbonates, but not alkaline bicarbonates, precipitate the free base from novocaine solutions as an oil that quickly becomes crystalline. This oil is insolu-

ble in water but readily soluble in alcohol, ether, benzene, and chloroform. The free base melts at 61–63°; novocaine at 155°. The picrate crystallizes in yellow needles melting at 156°. Ether easily extracts the free base from a solution rendered alkaline with sodium hydroxide solution.

Reactions

1. **Diazonium Reaction.**—Novocaine is a primary aromatic base and can be diazotized. The diazonium compound may then be coupled with phenols, thus forming azo-dyestuffs. Acidify an aqueous novocaine solution with hydrochloric acid. Then add a few drops of 0.5 per cent. sodium nitrite solution and finally β-naphthol dissolved in excess of sodium hydroxide solution. This produces a scarlet-red color. Cocaine, acoine, alipine, eucaine, holocaine, stovaine and psicaine do not give this reaction. The primary amine anaesthesine gives a strong and nirvanine a very weak color reaction. Detection of novocaine in presence of cocaine is based upon this diazonium reaction (see page 191).

2. **Calomel Test.**—Addition of a few drops of diluted alcohol to an intimate mixture of equal quantities of novocaine and calomel after some time causes a blackening to appear. Cocaine behaves in a similar manner.

3. **Iodoform Test.**—Cleavage of the ethyl group from novocaine takes place easily and consequently the odor of iodoform is apparent when the precipitate produced by iodo-potassium iodide is dissolved in sodium hydroxide solution and then gently warmed.

The following reactions serve to differentiate novocaine from cocaine (Gadamer):

(a) Add a few drops of 5 per cent. mercuric chloride solution to 0.1 gram of substance dissolved in 5 cc. of water. Novocaine gives a white precipitate soluble in a little dilute hydrochloric acid. Cocaine gives a white precipitate insoluble in a few drops of hydrochloric acid.

(b) Add a drop of 1 per cent. potassium permanganate solution to 0.25 gram of substance dissolved in 5 cc. of water. At first the novocaine solution remains clear and for a short time violet. Then with loss of color a separation of $MnO_2.H_2O$ takes place. Cocaine gives a violet precipitate of cocaine permanganate. Reduction takes place only after very long standing.

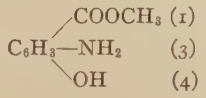
(c) Add 5 drops of 3 per cent. chromic acid solution to 0.05 gram of substance dissolved in 5 cc. of water. The color of the novocaine solution at first does not fade; later turbidity appears and fading

that is arrested by addition of hydrochloric acid. The precipitate in the cocaine solution at first continues to disappear and upon acidification remains as yellow, resinous cocaine chromate.

Physiological Action.—An aqueous novocaine solution (1 + 1) dropped upon mucous membranes, or injected subcutaneously, produces temporary but intense anaesthesia. Pupils, accommodation and blood vessels are unaffected. At the highest, the toxicity of novocaine is one-sixth as much as that of cocaine. Its action upon the nerve-roots is the same as that of cocaine.

ORTHOFORM (New)[1]

Orthoform is the methyl ester of meta-amino-para-oxy-benzoic acid:



This compound is a white or yellowish crystalline powder without odor or taste. Applied to the tongue it does not produce insensibility. Almost insoluble in water, it dissolves easily in 6 parts of alcohol, 50 parts of ether, as well as in benzene and chloroform. Orthoform at the same time a base and a phenol is dissolved both by acids and alkaline hydroxides. In aqueous solution its salts with acids have an acid reaction, an acidulous taste, and a weak anaesthetic action upon the tongue. Solutions in alkaline hydroxides turn red in the air from oxidation. The commercial product melts at 142°. Because of the feeble basic nature of orthoform its salts with acids are hydrolyzed to such an extent in aqueous solution that in the Stas-Otto process a very considerable quantity of the base can be extracted from a tartaric acid solution. Ether will not remove orthoform from a solution made alkaline with sodium hydroxide but extracts it easily from a solution rendered alkaline with sodium bicarbonate.

Reactions

1. **Diazonium Reaction.**—Orthoform is a primary aromatic amine and can be diazotized. A few drops of sodium nitrite solution turn a solution of orthoform in dilute hydrochloric acid greenish yellow. Addition of a strongly alkaline solution of β-naphthol produces a fine red color due to formation of an azo-dyestuff.

2. **Ferric Chloride Test.**—The violet color imparted by a little ferric chloride to an alcohol solution of orthoform becomes brown with more of the reagent and the aqueous filtered extract of it green.

3. **Nitric Acid Test.**—Addition of a drop of nitric acid to orthoform rubbed with a little concentrated sulphuric acid colors the mixture red-violet or blue-violet. Excess of sodium hydroxide solution changes this color to red.

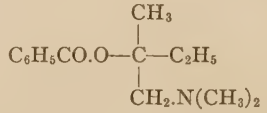
[1] "Orthoform Old" is isomeric with "Orthoform New."

4. Lead Peroxide Test.—Lead peroxide colors a glacial acetic acid solution of orthoform green.

Physiological Action.—In general, esters of aromatic oxy-amino-benzoic acids, such as orthoform, possess the property of causing local anaesthesia. On account of its insolubility, orthoform cannot be used subcutaneously. According to Kobert, the two orthoforms occasionally give rise to extensive necroses. They produce no anaesthesia upon unbroken and mucous surfaces of the body. The two orthoforms find no application in work upon the eye.

STOVAINE

Stovaine is the hydrochloride of the dimethylamino-ethyl-isopropyl ester of benzoic acid:



Stovaine resembles cocaine in having two atomic complexes that are the same, that is, a methylated tertiary amino-group and the benzoic acid ester of an alcohol. It is a white crystalline powder. Because of decomposition the faint odor of an amine base is apparent. Stovaine has a bitter taste and applied to the tongue causes temporary anaesthesia. It is very easily soluble in water and dissolves in 5 parts of methyl alcohol, 70 parts of absolute alcohol, and 50 parts of chloroform. In ether and acetone it is almost insoluble. It melts at 175–176°. The free base is a colorless oil dissolving very freely in alcohol and ether and almost insoluble in water. Ether extracts it from aqueous stovaine solutions that have been rendered alkaline with sodium hydroxide solution. The picrate of stovaine crystallizes well.

Reactions

General alkaloidal reagents give precipitates with aqueous stovaine solutions. Detection of stovaine depends mainly upon recognizing the presence of the benzoyl-group. Heat the material in a boiling water-bath for a few minutes with a few drops of alcohol and concentrated sulphuric acid. If stovaine is present, the odor of ethyl benzoate will be detected, especially if a little water is cautiously added to the mixture. The following reactions serve to differentiate stovaine from cocaine:

(a) Saturated aqueous picric acid solution gives with cocaine fine crystalline needles melting at 165–166°. Stovaine forms tabular crystals melting at 115–116°.

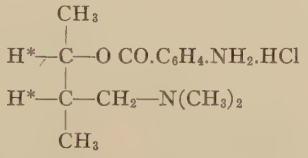
(b) Add 5 drops of 1 per cent. potassium permanganate solution to 0.1 gram of substance dissolved in 5 cc. of water. The violet

crystalline precipitate given by cocaine even after 0.75 hour retains its color unchanged. With stovaine the solution remains clear and after 0.75 hour the color disappears entirely with separation of $MnO_2.H_2O$.

Physiological Action.—Applied to mucous membranes stovaine produces intense local anaesthesia. It does not cause contraction of the blood-vessels. Following medicinal doses of 0.02–0.05 gram, the heart-beat is slowed but strengthened rather than weakened. According to Kobert, the toxic effects consist of tonic and clonic convulsions that may terminate in collapse. The lethal dose is said to be twice as great as that of cocaine. Introduction of 1 per cent. stovaine solutions into the eye produces not only anaesthesia but also slight enlargement of the pupil and accommodation disturbances. In the eye of the rabbit, 4 per cent. stovaine solution causes removal of the epithelium of the cornea (de Lapersonne). Following injection of 2 per cent. stovaine solution, Sinclair observed in 4 patients appearance of local gangrene.

TUTOCAINE

Tutocaine[1] is the hydrochloride of p-amino-benzoyl-α-dimethylamino-β-methyl-γ-butanol, that is, it is a derivative of an α-amino-γ-alcohol and has the following structure:



The presence of two asymmetric carbon atoms (*) makes possible several isomeric forms of tutocaine. The commercial product is a racemic compound, the hydrochloride of which melts at 213–215° and forms fine, white, microscopic needles that are soluble in water at 20° to the extent of 15.5 per cent. Its aqueous solution is colorless and neutral and during sterilization does not undergo decomposition. Tutocaine dissolves with difficulty in alcohol.

Reactions

1. Sodium hydroxide solution, added to an aqueous tutocaine solution warmed to about 50°, precipitates a yellowish oil.

2. Mercuric chloride produces a white and iodine solution a brown precipitate. Silver nitrate precipitates white silver chloride.

3. Azo-dye Test.—As a primary aromatic amine, tutocaine can be diazotized and converted into an azo-dye. Dissolve 0.1 gram of tutocaine in 5 cc. of water, add 2 drops of hydrochloric acid, and then 2 drops of sodium nitrite solution. Add this mixture to a solution of 0.2 gram of β-naphthol in 1 cc. of sodium hydroxide solution and 9 cc. of water. A scarlet-red precipitate is produced.

[1] W. Schulemann: Tutocaine as a Surface and Infiltration Anaesthetic. Klinische Wochenschrift III, No. 16.

4. **Permanganate-Sulphuric Acid Test.**—Add 3 drops of dilute sulphuric acid to a solution of 0.1 gram of tutocaine in 5 cc. of water and then 1 drop of dilute potassium permanganate solution. The violet color of the latter should at once disappear.

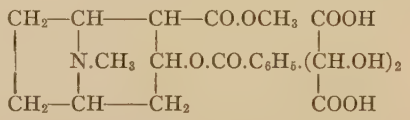
5. If 0.1 gram of tutocaine is dissolved in 1 cc. of nitric acid (25 per cent. HNO_3), the solution should have no color.

6. Hydrogen sulphide water added to a solution of tutocaine in water should produce no change.

Physiological Action.—In a rabbit's or man's eye, a tutocaine solution causes no irritation whatever. A 2 per cent. solution moderately enlarges the blood-vessels of the conjunctiva of the human eye. Slight enlargement of the pupil of the human eye appeared some time after the solution had been introduced. A 1–0.25 per cent. solution of tutocaine introduced into the conjunctival sac of a rabbit's eye produced complete anaesthesia of the cornea in about 4 minutes. The action of tutocaine is very persistent. Where a 1 per cent. tutocaine solution was injected in 2 minutes into the auricular vein of the rabbit, the lethal dose was 25–30 mg. Following entrance of tutocaine into the circulation, toxic effects decrease as elimination of the poison takes place which proceeds rapidly in the human organism. In toxicity tutocaine is very close to novocaine. Unlike cocaine it exhibits no tendency toward cumulative action.

PSICAINE

Psicaine[1] is the acid tartrate of dextro-rotatory pseudo-cocaine, or dextro-ψ-cocaine, having the composition $C_{17}H_{21}O_4N.C_4H_6O_6$ and the structural formula:



Psicaine is a white crystalline powder soluble in 4 parts of water and with somewhat more difficulty in alcohol. Aqueous psicaine solutions are acid to litmus, have a bitter taste, and applied to the tongue produce prolonged insensibility. They remain unchanged when sterilized for one hour in a current of steam. Psicaine may be differentiated from ordinary cocaine by its behavior toward polarized light, $[\alpha]_D^{20} = +43°$. In the course of his cocaine synthesis, Willstätter[2] examined dextro-pseudo-cocaine.

Pharmacological Properties and Uses in Medicine.—Anaesthetic strength is influenced by spatial arrangement of atoms in the cocaine molecule and is increased in the pseudo-series. The activity of psicaine, when brought into contact with peripheral nerve endings, is about twice that of ordinary cocaine. The organism moreover rids itself more easily of psicaine than of cocaine. Animals tolerate twice as much psicaine subcutaneously injected as cocaine without intoxi-

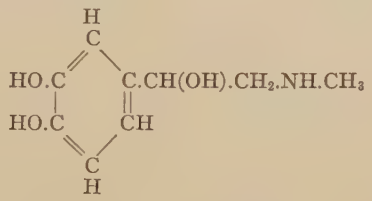
[1] R. Gottlieb: Archiv f. experim. Path. u. Pharmakol. 97 (1923), 113; Münchener med. Wochenschr. 26 (1924), 850.

[2] R. Willstätter: Münchener med. Wochenschr. 26 (1924), 849; R. Willstätter, O. Wolfes and H. Maeder: Annalen d. Chem. 434 (1923), 111.

cation. Recovery is more rapid from psicaine than from cocaine intoxication. Except for traces eliminated in the urine, psicaine is entirely destroyed in metabolism, whereas cats eliminate 5–20 per cent. of cocaine. Otherwise the pharmacological behavior of psicaine is quite like that of cocaine. Like the latter it penetrates well. At first there is enlargement of the blood vessels but addition of adrenaline promptly produces contraction. Psicaine behaves well in conjunction with adrenaline. In small doses cocaine exhibits euphoretic action, whereas psicaine does not. Consequently appearance of cocainism is excluded when psicaine is used. Psicaine is used principally as a superficial anaesthetic, especially upon mucous surfaces.

SUPRARENINE OR ADRENALINE

Suprarenine, adrenaline, or epinephrine, the active substance of the suprarenal gland, causing contraction of blood-vessels and increase of blood-pressure, is ortho-dioxy-phenyl-ethanol-methyl-amine having the following structure:



The laevo-rotatory form of adrenaline occurs in the suprarenal gland. This alone finds use in medicine. Its physiological action is manifested in pronounced contraction of blood-vessels, great increase of blood-pressure, slowing of heart action, enlargement of pupils, and increased excretion of urine. Glycosuria may also appear. In addition to changes in pulse, blood-pressure and pupils, Kobert cites a case observed by Ambey in which, after intravenous injection of adrenaline, appeared vomiting, excitement, weakness, bloody diarrhoea and death during the picture of prostration.

Laevo-adrenaline is a white crystalline powder easily turning gray in air, or small white needles that decompose at 212°. It has a faintly bitter taste and produces insensibility when applied to the tongue. It is difficultly soluble in water with alkaline reaction, even less soluble in alcohol, and practically insoluble in ether, benzene and chloroform. At the same time a diatomic phenol and a di-acid base, it dissolves both in alkaline hydroxides and acids $[\alpha]^{D}_{19.6°} = -51.4°$.

Synthetic Adrenaline.—Catechol gently heated with monochlor-acetic acid in presence of phosphorus oxychloride is converted into chlor-aceto-catechol. The

latter, kept cold and treated with methyl-amine, is converted into methyl-amino-aceto-catechol. This compound is a ketone and can be reduced to the secondary alcohol, adrenaline:

$$C_6H_3(OH)_2H + HOOC.CH_2Cl \rightarrow C_6H_3(OH_2).CO.CH_2Cl + NH_2.CH_3 \rightarrow$$

Catechol — Chlor-aceto-catechol

$$C_6H_3(OH)_2.CO.CH_2.NH.CH_3 + 2H \rightarrow C_6H_3(OH)_2.CH(OH).CH_2.NH.CH_3$$

Methyl-amino-aceto-catechol — Adrenaline

Synthetic adrenaline obtained in this manner is inactive. It is a white crystalline powder that decomposes at about 230°. By fractional crystallization of the acid dextro-tartaric acid salt, Flächer[1] succeeded in resolving synthetic dl-suprarenine into its two components. In physical, chemical and physiological properties synthetic l-suprarenine is identical with the product isolated from suprarenal glands. The effect of l-suprarenine upon blood-pressure is much greater than that of d-suprarenine. E. Abderhalden and Fr. Müller[2] have shown that l-suprarenine is about 15 times more active than d-suprarenine. Corresponding to its composition, dl-suprarenine occupies a position midway between the two.

Detection

Suprarenine as a derivative of catechol possesses in general the chemical properties of this compound. It is easily oxidized and consequently is a powerful reducing agent. Suprarenine gives the well-known reduction tests with ammoniacal silver nitrate, gold chloride solution, and with Nessler's reagent. Iodic acid is reduced. Therefore a mixture of suprarenine and iodic acid colors chloroform violet. A mixture of ferric chloride and potassium ferricyanide turns blue from formation of Prussian blue. Phospho-molybdic acid gives a faint green color changing to deep blue with excess of sodium hydroxide solution. This blue color appears especially well if the solution is acidified with hydrochloric acid. Even very dilute adrenaline solutions are colored deep blue by phospho-tungstic acid with addition of excess of saturated sodium carbonate solution. This test is also a reaction of reduction, for under like conditions various reducing substances, such as uric acid, also give blue solutions with phospho-tungstic acid.

Ferric Chloride Test.—Very dilute ferric chloride solution imparts an emerald-green color to a solution of adrenaline, as it does to a catechol solution. Ammonia changes this color to blood-red.

[1] F. Flächer: Cleavage of Synthetic dl-Suprarenine into Its Optically-active Components. Zeitschr. f. physiol. Chem. 58 (1908), 189.

[2] E. Abderhalden and Fr. Müller: Behavior of Blood Pressure after Intravenous Injection of l-, d- and dl-Suprarenin. Zeitschr. f. physiol. Chem. 58 (1908), 185.

Concentrated sulphuric acid dissolves adrenaline with yellowish color changed to yellow-red by a drop of 25 per cent. nitric acid. Marquis' reagent dissolves it first with rose-red color that changes later to cherry-red. Froehde's reagent dissolves it first brown and then greenish. Millon's reagent dissolves it with yellow-red color (Kraus[1]).

Estimation of Adrenaline According to Folin[2] in the Suprarenal Gland with the Autenrieth-Königsberger Wedge-Colorimeter[3]

Weigh finely comminuted glands freed from fat and triturate well in a mortar with fine sand and 0.1 n-hydrochloric acid. Then transfer this mixture without loss to an Erlenmeyer flask, rinsing out the mortar with 0.1 n-hydrochloric acid and water. For every 2 grams of fresh suprarenal glands use a total of 15 cc. of acid and about three times as much water. Heat the mixture to boiling. No coagulation of albumin takes place in presence of so much hydrochloric acid but adrenaline is dissolved. Add to the boiling liquid 5 cc. of 10 per cent. sodium acetate solution for every 15 cc. of 0.1 n-hydrochloric acid and continue boiling. This will produce a precipitate containing albumin. Pour the entire mixture, with exception of the sand, into a graduated flask holding 100 cc. for 2 grams of gland, 200 cc. for 2–4 grams, and 500 cc. for 8–10 grams. Cool and fill to the mark with water, rinsing out the Erlenmeyer flask with water. Shake well and pour through a dry filter. Estimate the adrenaline content and for the colorimetric determination put 5–10 cc. of the clear filtrate into a 50 cc. graduated flask, adding 1 cc. of phospho-tungstic reagent and 10 cc. of saturated sodium carbonate solution. Allow the mixture to stand for 10 minutes, fill to the mark with water and shake. By means of a calibrated comparison-wedge determine in the colorimeter mentioned above the tinctorial strength of the blue solution obtained. Fill the comparison-wedge with an artificial color solution that keeps and is optically identical with the blue adrenaline solution to be examined. In this case, for example, a solution of copper tetramine sulphate may be used.

Calibration of the Comparison-Wedge

Calibrate this wedge in the following manner with "Suprareninum crystallisatum purissimum"[4] supplied in small tubes holding 0.05 gram. Dissolve the contents of such a tube in a little dilute hydrochloric acid and then dilute this solution to 500 cc. with water. 1 cc. = 0.1 mg. of adrenaline. Put 1, 2, 3, etc., cc. of this solution in a 50 cc. graduated flask, adding in each case 1 cc. of

[1] L. Kraus: Certain Color Reactions of Synthetic Suprarenine. Apoth.-Ztg. 23 (1908), 701.

[2] O. Folin, W. B. Cannon and W. Dennis: Colorimetric Method of Estimating Suprarenine. The Journ. of Biol. Chem. 13 (1912/13), 477.

[3] W. Autenrieth and H. Quantmeyer: Estimation of Adrenaline in the Suprarenal Capsule and of Uric Acid in Blood. Münch. med. Wochenschrift 1921, No. 32, 1007.

[4] A preparation of the Höchst Color Works, formerly Meister, Lucius and Brüning.

phospho-tungstic acid reagent and 10 cc. of saturated sodium carbonate solution. Allow the mixture to stand for 10 minutes, fill to the mark with water and shake. Determine in the colorimeter for each color solution the point at which it is identical with the comparison-wedge in color strength. The following values were determined in this manner:

Adrenaline solution in cc.....	1.0	1.5	2.0	2.5	3.0
Adrenaline in mg.....	0.1	0.15	0.2	0.25	0.3
Reading for equal color strength	74	64	53	43	33

In each case, record adrenaline in mg. upon the abscissae and readings for equal color strength upon the ordinates of a coordinate system. The line connecting points of intersection of coordinates is the calibration curve of the comparison-wedge. With this comparison-wedge, if in each case the color solution is diluted to 50 cc., it is possible to determine quantities of adrenaline from 0.05 to 0.45 mg.

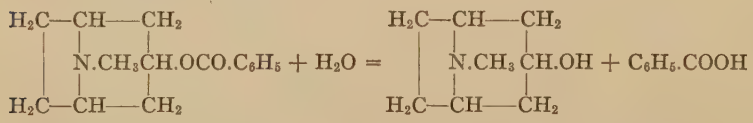
Example.—Weight of the two lobes of the suprarenal glands of the ox = 12.3 grams. Extract solution = 600 cc. Blue color solution prepared from 5 cc. of this solution = 50 cc. Equal color strength at 51 on the scale = 0.26 mg. of adrenaline. So the adrenaline content of the extract and of the entire suprarenal gland: $120 \times 0.26 = 31.2$ mg.

Preparation of Phospho-tungstic Acid Reagent

Heat a mixture of 100 grams of sodium tungstate with 80 cc. of 85 per cent. phosphoric acid and 750 cc. of water to boiling under a reflux. Boil for 4 hours, cool and make up to a liter with water. This reagent should be kept in small dark bottles.

TROPACOCAINE

Tropacocaine, $C_{15}H_{19}NO_2$, is benzoyl-pseudo-tropeine occurring in Java coca leaves together with cocaine. The free base forms white shining plates melting at 49°. It dissolves with great difficulty in water but readily in alcohol, ether, chloroform and benzene. Aqueous solutions of tropacocaine have a strong alkaline reaction. As a rule salts of tropacocaine with one equivalent of acid crystallize well. The hydrochloride, $C_{15}H_{19}NO_2.HCl$, is used in medicine. Solutions of tropacocaine are optically inactive. Heated with concentrated hydrochloric acid, tropacocaine is decomposed into benzoic acid and pseudo-tropeine:



Pseudo-tropeine is stereo-isomeric with tropine. It melts at 108° and dissolves readily in water and alcohol but with more difficulty

in ether. Tropine melts at 63° and dissolves easily in water, alcohol and ether. Both are strong bases and boil without decomposition.

Reactions and Detection

In many respects tropacocaine is much like cocaine. Sodium hydroxide solution added to solutions of its salts causes milkiness and precipitates the amorphous base easily soluble in ether. Consequently in the Stas-Otto process tropacocaine will appear in the ether extract of the aqueous solution after it has been made alkaline with sodium hydroxide solution. Evaporation of the ether solution leaves tropacocaine as an oily residue. If the latter is warmed upon the water-bath for a short time to expel moisture, it becomes crystalline as it cools. Most of the general alkaloidal reagents added to solutions of tropacocaine salts produce abundant precipitates. More than traces of tropacocaine may be identified by some of its well crystallized salts and thus distinguished from cocaine. Tropacocaine hydrochloride crystallizes in leaflets melting with decomposition at 276–277°, whereas cocaine hydrochloride heated slowly melts sharply at 182–183°.

Saturated picric acid solution forms a difficultly soluble precipitate crystallizing from hot water containing a little alcohol in needles and leaflets that darken at 215–220° and melt at 240–242°. Cocaine picrate melts at 165–166°.

Tropacocaine chloraurate, $C_{15}H_{19}NO_2.AuCl_4$, is formed even in extreme dilution and crystallizes from hot water without decomposition in yellow needles melting at 208° with decomposition, whereas cocaine chloraurate, $C_{17}H_{21}NO_2.AuCl_4$. is obtained in the form of a light yellow amorphous precipitate from which metallic gold easily separates.

Potassium dichromate, added to a 0.5 per cent. solution of tropacocaine hydrochloride, at once produces a thick crystalline precipitate that dissolves with some difficulty upon heating the liquid and again separates in compact crystals as the solution cools. The same reagent added to the corresponding cocaine solution produces only after acidification with a little hydrochloric acid a resinous precipitate that coalesces in oily drops when the liquid is warmed. As the liquid cools it again separates resinous and becomes finely crystalline only after long standing, usually after several days.

Physiological and Pharmacological Properties

The physiological behavior of tropacocaine is similar to that of cocaine. In anaesthetic action it is more rapid than cocaine and also less toxic. Anaesthesia from tropacocaine, however, is not as prolonged as that from cocaine. According to Kochmann,[1] the toxicity of tropacocaine is 8 times less than that of cocaine. In case of tropacocaine the ratio of local anaesthetic power to toxicity is more favorable than in that of cocaine. Small doses cause no enlargement of the pupil and even when it can be detected is very slight. Tropacocaine gives rise to no disturbances of accommodation and causes no increase of blood-pressure, since it enlarges the blood-vessels. In consequence of its action in enlarging blood-vessels, tropacocaine is not adapted for use in conjunction with preparations of the suprarenal glands or with adrenaline. According to Braun,[2] tropacocaine is useful in several ways for purposes of local anaesthesia. When anaesthesia of long duration is not desired, tropacocaine on account of its non-toxicity and freedom from irritation is to be preferred to cocaine. In ophthalmological work a 5 per cent. solution appears to be specially serviceable. It causes anaesthesia of the cornea and conjunctiva in a few seconds which is amply sufficient for the removal, for example, of foreign bodies. In this case tropacocaine is a better anaesthetic than cocaine, for it acts more quickly, leaves the cornea unchanged, and avoids intra-ocular rise of blood-pressure as well as mydriasis. Addition of salt to the tropacocaine solution lessens any tendency to cause enlargement of blood-vessels and reddening of the conjunctiva. Tropacocaine is especially useful in producing anaesthesia of the spinal cord. In anaesthesia of the lumbar region it is essential that the anaesthetic used exert as little toxic action as possible upon the nerve roots and their nuclei. Experiments upon animals have shown that of the substances in the group comprising cocaine and cocaine-substitutes tropacocaine comes nearest to fulfilling this requirement. Following intralumbar administration of tropacocaine, anaesthesia appears after 3–4 minutes and lasts as a rule for 45–60 minutes. The average dose is 0.5–0.6 gram of tropacocaine hydrochloride. Injurious effects upon heart, lungs and kidneys caused by lumbar anaesthesia from tropacocaine are unknown. The most common after-effect is headache in the frontal region or occiput which may be of long or short duration and appear immediately or in a few days after the drug has been used. Very large doses of tropacocaine cause rise of temperature and outbreak of convulsions. Death ensues from respiratory paralysis (Kobert).

LOBELINE

Lobelia inflata, indigenous to North America, contains several active alkaloids that can be isolated from the plant by extraction with alcohol. Neutralization of the residue from this extract with acid, usually dilute sulphuric acid, converts it into a brown-yellow resinous mixture of salts known commercially as "Lobeline." The effects of very small subcutaneous doses of this lobeline recall apomorphine (central emesis), those from small internal doses and from smoking the leaves of Lobelia inflata recall nicotine, and those from large doses recall

[1] M. Kochmann: On Anaesthetics. Therapeutische Monatshefte 1914.

[2] H. Braun: Local Anesthesia, Its Scientific Foundations and Practical Application. Third Edition.

coniine, since curare-like paralysis appears in frogs. In man the peripheral ends of the lung vagus are paralyzed by lobeline. Consequently asthmatic contractions of the bronchiae are allayed. For this reason officinal tincture of lobelia is specific for asthmatic pains. A dose of 11 mg. of lobeline resulted in marked somnolence and diarrhoea. Vomiting did not appear. Following administration of large doses of lobeline to dogs and cats, muscular twitching and tonic convulsions appeared. Death ensued from paralysis of the respiratory centre.

Wieland[1] has isolated several alkaloids in pure crystalline form from Lobelia inflata. The most important compound is known as lobeline. To distinguish this product from commercial lobeline, the author suggests that this pure preparation be called "Lobelinum crystallisatum Wieland." Pure lobeline has the composition $C_{23}H_{29}NO_2$. It is a monacid base that appears in beautiful colorless crystals, melting at 128–129°, and forms well crystallized salts having a neutral reaction. Wieland has ascertained the following facts with regard to the constitution of lobeline. Quite probably the two oxygen atoms are in ether-like combination, since reagents for carbonyl and hydroxyl give no evidence of reacting. Methoxyl groups are not present. A lactone group also is probably not present, since lobeline is stable in presence of solutions of caustic alkalies. Heated with water, lobeline is decomposed into acetophenone, isolated to the extent of more than 60 per cent. of the weight calculated for one molecule, and a basic product having the composition $C_{15}H_{21}NO$ or $C_{15}H_{23}NO_2$.

The observation of Wieland that chloroform extracts lobeline hydrochloride from aqueous solution, while leaving the resinous salts of the other bases dissolved in water, is the basis of the isolation of crystallized lobeline. Faintly acidify the solution of the crude lobelia alkaloids with hydrochloric acid and make about 10 extractions, shaking vigorously each time with $\frac{1}{20}$ the volume of chloroform. Evaporation of the combined chloroform extracts, finally *in vacuo*, leaves a light brown syrup. Repeatedly digest the latter with water at 60° for 10 minutes each time. By allowing the filtered extracts to stand in a vacuum desiccator over solid potassium hydroxide and concentrated sulphuric acid, the hydrochloride is obtained in crystals.

The free lobeline base may be obtained by quickly dissolving the combined residues from crystallization in warm water, thoroughly cooling the solution, and decomposing lobeline hydrochloride in a separating funnel under ether with a slight excess of sodium hydroxide solution. Ether at once dissolves the free base. Dry the ether solution with potash, distil off most of the ether, and allow the concentrated solution to evaporate slowly. Crystals soon begin to appear. The crude base may be recrystallized from alcohol, benzene or ether. Pure lobeline dissolves in water and petroleum ether with great difficulty but is freely soluble in chloroform, hot benzene and hot alcohol. A cold saturated alcoholic solution contains 3.7 per cent. of alkaloid. Lobeline is only slightly soluble in ether.

Lobelidine, $C_{20}H_{25}NO_2$, melting at 106°, is another crystalline lobelia alkaloid obtained by Wieland. In appearance pure lobelidine is not unlike lobeline but widely different from it in composition, melting-point and its hydrochloride.

[1] H. Wieland: Alkaloids of Lobelia I. Berichte d. Deutsch. chem. Ges. 54 (1921), 1784.

Detection

Since lobeline is a strong base, it is obtained in the Stas-Otto process when the aqueous solution made alkaline with sodium hydroxide or sodium carbonate solution is extracted with ether. Evaporation of solvent leaves an amorphous residue having a faint odor of the lobelia plant, provided poisoning was due to the plant. In this case the solution of this residue in water containing acid gives precipitates with potassium mercuric iodide, potassium bismuthous iodide, and phospho-molybdic acid. From solutions of lobeline salts, mercuric chloride precipitates difficultly soluble amorphous double salts (Wieland).

Impure lobeline, obtained from material after poisoning due to the plant or tincture, gives with Froehde's reagent after about 2 minutes a violet color that increases in intensity during a period of 1–2 hours and later changes to brown or yellow (Gadamer).

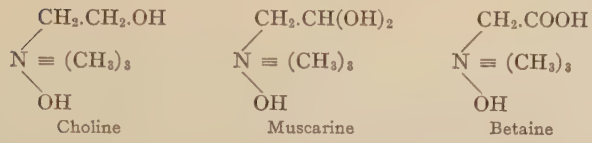
Behavior of Pure Lobeline toward Reagents

Lobeline dissolves in concentrated sulphuric acid without color. A very faint reddish color gradually appears and later becomes faintly brownish. Solution in concentrated nitric acid is also colorless. Marquis' reagent gives a deep violet to cherry-red solution. These colors appear immediately and are very stable. Froehde's reagent first gives a nearly colorless solution that gradually changes to faint rose-red and later has a greenish brown border. Mecke's reagent behaves like concentrated sulphuric acid. Mandelin's reagent gives a not very characteristic brown color with violet streaks and later becomes greenish. Picric acid gives a yellow crystalline precipitate that may be recrystallized from hot water. Picrolonic acid gives amorphous greasy precipitates. Chloro-platinic acid gives a light yellow precipitate consisting of small microscopic prisms. Potassium bismuthous iodide gives a precipitate consisting of compact red-brown crystals. Iodic acid is not reduced.

Wieland brings about the cleavage of acetophenone ($C_6H_5.CO.CH_3$) by heating 2 grams of pure lobeline with 20 cc. of water for 6 hours at 110° in sealed tube. The contents of the tube are dissolved in ether and the ether solution extracted with dilute hydrochloric acid to remove basic substances. The ether is distilled and the residue subjected to steam distillation. Acetophenone passes over leaving almost no residue. The distilled acetophenone is again dissolved in ether and finally weighed in a tared flask. The yield is 0.43 gram = 60 per cent. of the quantity theoretically possible for the cleavage of 1 molecule of acetophenone. The loss is to be attributed to volatilization. For identification acetophenone was converted by an alcoholic solution of o-nitro-phenyl-hydrazine into its o-nitro-phenyl-hydrazone, $\begin{matrix} CH_3 \\ C_6H_5 \end{matrix}\!\!>\!C = N\text{—}NH.C_6H_4.NO_2$, that crystallizes in beautiful orange-red needles melting at 143°.

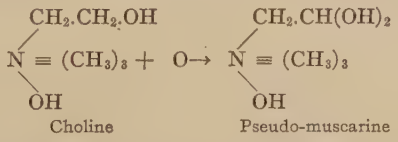
MUSCARINE

Muscarine, or oxycholine, $C_{15}H_{15}NO_3$, is closely related chemically to choline and betaine which are fairly widespread in the plant kingdom:



Muscarine was discovered in 1869 by Schmiedeberg and Koppe[1] in the fly mushroom, Amanita muscaria, together with other substances, particularly choline. To distinguish the natural product from other synthetically prepared muscarines, it is given the more precise designation "fly mushroom muscarine." That the latter is formed in the fly mushroom as a result of the oxidation of the abundant choline appears to be not entirely improbable. The statement made by different observers that all fly mushrooms do not contain muscarine is worthy of note. Northern fly mushrooms are said not to contain preformed muscarine. At least Nencki and Berlinerblau failed to find muscarine in the alcoholic extract of Siberian fly mushroom. It is first formed when the mushroom is boiled with water. The presence of muscarine, as shown by Brieger, in putrid codfish deserves mention. In this case also the alkaloid probably comes from choline always present in the flesh of fish. Muscarine also occurs in other mushrooms, such as the Panther mushroom, Amanita pantherina, Boletus luridus, and Russula emetica, according to R. Kobert.

Properties.—As usually obtained, muscarine is an inodorous, tasteless syrup without color when pure. It is deliquescent in air and has a strong alkaline reaction. It dissolves easily in water and alcohol but is nearly insoluble in ether and chloroform. Consequently muscarine cannot be extracted in the Stas-Otto process. Moreover it cannot be extracted from aqueous solution by benzine, benzene, ligroin or petroleum ether. Muscarine and its salts when warmed with potassium hydroxide solution give off trimethylamine, $N(CH_3)_3$, recognized by its fish-like odor. Choline, neurine and neuridine behave in the same way. A base containing one more atom of oxygen is formed by oxidation of choline, that is, by boiling its hydrochloride or its chloro-platinate with nitric acid. This compound has the same chemical composition as muscarine from fly mushroom but different physiological properties and consequently cannot be identical with the latter. This synthetic muscarine is called choline-muscarine or pseudo-muscarine:



Muscarine is characterized by its physiological action and by the appearance of its chloro-platinate, $(C_{15}H_{14}NO_2)_2PtCl_6.2H_2O$, as difficultly soluble octahedrons or yellow needles and of its chlor-aurate, $C_{15}H_{14}NO_2.AuCl_4$, difficultly soluble in cold water.

Physiological Properties.—The most striking symptom of the action of pure muscarine is its actual or apparent irritation of the cardio-inhibitory center

[1] O. Schmiedeberg and R. Koppe: Muscarine, the Poisonous Alkaloid of Amanita Muscaria. Leipsic, 1869.

sufficiently strong to produce complete stasis of the heart in no wise paralyzed. Many pharmacologists hold the view that muscarine by its action upon the heart shows itself to be a pure muscle poison causing paralysis.. This stasis of the heart in diastole may cause death at once in man and mammals. Straub has furnished evidence that the heart is rendered incapable of functioning only when muscarine has penetrated the muscle fibres. The action of atropine upon the vertebrate heart is said to depend upon the fact that it diminishes the speed with which muscarine collects. Fungus muscarine stimulates all vertebrate peripheral apparatus and organs paralyzed by atropine. Consequently atropine almost instantly allays in vertebrates the effects of fungus muscarine. Previous administration of atropine will prevent appearance of the effects of muscarine. Atropine in its action is therefore markedly antagonistic to that of muscarine. The statements made by Schmiedeberg with regard to the symptoms produced by very small doses of muscarine are: lower frequency of pulse, fall of blood-pressure, salivation, flow of tears, increased pancreatic, biliary, mucous, spermatic and sweat secretion, contraction of pupils, accommodation spasms, and violent contractions of stomach and intestinal tract resulting in emesis and diarrhoea. In consequence of profuse secretion of intestinal glands stools may be watery and in the end bloody. As a result of the irritation of all glands the blood is thickened as in cholera and consequently the number of red blood-corpuscles in a unit volume of blood shows a marked increase. Finally contraction of bladder and uterus may occur. The effects in man following subcutaneous injection of 1–3 mg. of muscarine are: flow of saliva, rush of blood to the head, hence flushing of the face, increase in pulse frequency, nausea, rumbling in the abdomen, usually contraction of pupils, disturbances of vision, especially accommodation spasms, profuse perspiration on the face, and to a less degree on the rest of the body. In larger doses muscarine causes slowing of the pulse, vomiting, diarrhoea, convulsions, unconsciousness, and death due primarily to stasis of the heart (Kobert).

Use of the fly mushroom in northern lands as a beverage is of interest, that is, as a means of intoxication. Used for this purpose this fungus is said to produce typical intoxication accompanied by a condition of exhilaration and dilatation of pupils. Flow of saliva, emesis and diarrhoea, and pronounced slowing of the pulse are entirely lacking. A notable fact in this case is that subcutaneous injection of atropine, which easily allays all effects of intoxication arising from muscarine, does not end intoxication caused by drinking the beverage made from fly mushroom.

Detection and Isolation of Muscarine

Muscarine is quantitatively precipitated by mercuric chloride and potassium bismuthous iodide. Potassium mercuric iodide causes precipitation only when the reagent is added carefully and without excess of potassium iodide. Bromine water, chlor-auric acid, tannic acid, phospho-molybdic acid and phospho-tungstic acid also precipitate muscarine. The chlor-aurate and chloro-platinate of muscarine which crystallize beautifully probably suffice to characterize this base chemically (see above).

The method used by Brieger[1] in examining cadaveric material for ptomaines is especially adapted for isolating muscarine from organs and other parts of the cadaver. The material is comminuted and boiled with water containing a little dilute acid. The water should always show an acid reaction and boiling should not last longer than a few minutes. Evaporate the hot filtered solution upon the water-bath to a thick syrup. Gradually stir into the latter a considerable quantity of 96 per cent. alcohol. Many substances such as protein bodies and inorganic salts remain undissolved. Filter this solution and while it is still warm add an alcoholic solution of lead acetate avoiding an excess. This lead precipitate contains no bases and is filtered off. Evaporate the filtrate to a syrup and again take this up in 96 per cent. alcohol. Remove alcohol from this solution by evaporation or distillation. Dissolve the residue in water and remove lead by hydrogen sulphide. Warm to expel excess of hydrogen sulphide from the filtrate and precipitate with potassium mercuric iodide saturated with mercuric iodide. Most of the muscarine that may be present is precipitated. Filter off the precipitate, triturate with barium hydroxide, and decompose with hydrogen sulphide. Free the filtrate from barium with sulphuric acid and, having expelled hydrogen sulphide, remove iodine with silver chloride. The filtrate thus obtained after concentration is treated with chloro-platinic acid. Upon slow evaporation muscarine chloro-platinate crystallizes in octahedrons and needles that may be separated mechanically from red-yellow monoclinic plates of choline chloro-platinate. Evaporation of muscarine chloro-platinate solution with potassium chloride solution and extraction with alcohol yields muscarine hydrochloride, the alcoholic solution of which is evaporated. The residue taken up in water may be used for the physiological detection of muscarine.

Like potassium salts, for example, muscarine causes diastolic stasis of the frog's heart and is characterized by the fact that it can be counteracted by injection of atropine. In systolic stasis caused by substances acting like digitalis, the ventricle of the heart in consequence of maximal contraction appears pale, whereas in diastolic stasis at maximal expansion it appears dark. Addition of a drop of about 0.5 per cent. atropine sulphate solution to the ventricle brought to stasis by muscarine soon causes reappearance of regular heart-beats and after that the action of the heart appears normal as before. Consult H. Führer. "Nachweis and Bestimmung der Gifte auf biologischem Wege (1911), page 77 (Biological Detection and Estimation of Poisons).

Notes.—J. Gadamer ("Chemische Toxicologie') precipitates the faintly acid alcoholic extract of material, freed from alcohol, by lead acetate and ammonia in slight excess instead of an alcoholic lead acetate solution.

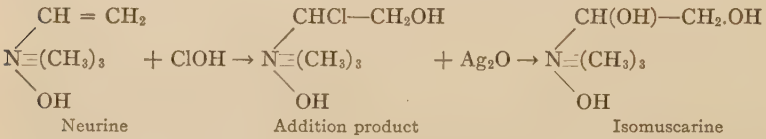
Synthetic Muscarines

Pseudo-muscarine, or muscarine from choline (see above), obtained by Schmiedeberg and Harnack[2] by oxidizing choline, has the same chemical composition as muscarine from fly mushroom but a different physiological action. It is there-

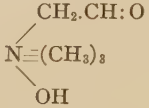
[1] L. Brieger: Researches upon Ptomaines, III Part. Berlin 1886.

[2] O. Schmiedeberg and E. Harnack: Synthesis of Muscarine and Muscarine-like Acting Ammonium Bases. Archiv f. experim. Pathol. u. Pharmak. 6 (1877), 101.

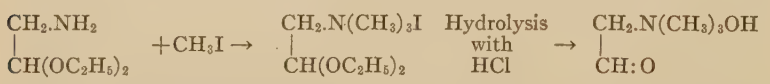
fore not identical with the latter. Isomuscarine of Bode and Schmidt[1] has strong toxic properties which are widely different from those of muscarine from fly mushroom. The constitution of isomuscarine is shown by its formation from neurine by the action of moist silver oxide upon its addition-product with hypochlorous acid:



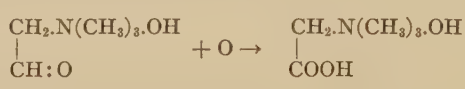
Neurine — Addition product — Isomuscarine

A fourth muscarine is to be regarded as betaine aldehyde, 

It was first obtained by Berlinerblau[2] from chlor-acetal and trimethylamine. Later E. Fischer methylated acetalamine and produced the corresponding aldehyde by hydrolysis of acetal-trimethyl-ammonium chloride with warm concentrated hydrochloric acid:



This aldehyde-ammonium base is not identical with muscarine. Its physiological action, according to Schmiedeberg, is entirely different from that of muscarine from fly mushroom and is more suggestive of that of choline. In conformity with its structure as betaine aldehyde, E. Fischer[3] succeeded in oxidizing this substance with silver oxide and obtaining the carboxylic acid betaine:



According to Nothnagel,[4] betaine aldehyde, which may be regarded as anhydro-muscarine, in quantities up to 0.01 gram has not the slightest effect upon the action of the frog's heart. This base also has no action upon the cat's eye nor upon the vagus apparatus controlling arrestment of action of the mammalian heart. On the other hand, anhydro-muscarine like most ammonium bases gives rise to secretion of saliva and sweat in great abundance. Death in mammals is brought about by paralysis of respiration.

[1] E. Schmidt: Choline, Neurine and Related Compounds. Annalen der Chemie 267 (1892), 249. J. Bode: Certain Derivatives of Neurine and Choline. Ibid. 267 (1892), 268.

[2] J. Berlinerblau: Muscarine. Berichte d. Deutsch. chem. Ges. 17 (1884), 1139.

[3] E. Fischer: Amino-Acetaldehyde. Ibid. 26 (1893), 464 and 27 (1894), 165.

[4] G. Nothnagel: Muscarine. Ibid. 26 (1893), 801.

DERIVATIVES OF MORPHINE

(Dionine, Heroine, Oxydimorphine)

DIONINE

Dionine, or ethyl-morphine hydrochloride, $C_{17}H_{17}O(OH)(OC_2H_5)N.HCl.H_2O$, a substitute for codeine, is a white, inodorous, micro-crystalline powder having a bitter taste and melting at 123–125°. It dissolves easily in water and alcohol with neutral reaction but is insoluble in ether and chloroform. Most of the alkaloidal reagents precipitate dionine from pure aqueous solutions even in extreme dilution. In its color reactions dionine differs hardly at all from codeine. Slight differences appear only in its behavior toward ammonia. Free codeine, precipitated by a few drops of ammonia (sp. gr. 0.91 = 25 per cent.) from 5 cc. of a 10 per cent. solution of codeine hydrochloride, remains permanently dissolved upon further addition of 1 cc. of ammonia. Free ethyl-morphine base precipitated under the same conditions is first temporarily dissolved by 5 cc. of ammonia and after a short time again separates in crystalline form. Free ethyl-morphine, $C_{17}H_{17}O(OH)(OC_2H_5)N.H_2O$, melts at 93° and crystallizes in shining prisms readily soluble in alcohol, ether and chloroform, less easily in benzene, and soluble in 280 parts of water.

Marquis' reagent first colors dionine distinctly green, then blue, and finally a fine blue-violet. Mecke's reagent at once colors it olive-green. This color upon gentle warming changes, sometimes through blue, to blue-green. The same reactions that distinguish codeine from morphine apply to dionine, especially its great solubility in ether and chloroform.

Dionine has proved efficient as an anodyne and soporific.

HEROINE

Heroine, or diacetyl-morphine, $C_{17}H_{17}ON(O.CO.CH_3)_2$, finds use as the free base and particularly in the form of the hydrochloride, $C_{17}H_{17}O(O.CO.CH_3)_2N.HCl$, as a substitute for codeine on account of its specific sedative action upon the respiratory organs. It is, however, more toxic than morphine.

Preparation.—Heroine is prepared by heating finely powdered anhydrous morphine with acetyl chloride upon the water-bath. From the hydrochloride thus formed the pure base is precipitated by sodium carbonate and recrystallized from acetic ether:

(a) $C_{17}H_{17}NO(OH)_2 + 2CH_3.COCl = C_{17}H_{17}NO(O.CO.CH_3)_2.HCl + HCl$

(b) $2C_{17}H_{17}NO(O.CO.CH_3)_2.HCl + Na_2CO_3 = 2C_{17}H_{17}NO(O.CO.CH_3)_2 + 2NaCl + CO_2 + H_2O$

Free heroine base crystallizes in white prisms having an alkaline reaction and a faintly bitter taste. It melts at 176–178°, is almost insoluble in water, difficultly soluble in ether and cold alcohol, and easily soluble in hot alcohol, chloroform and benzene. Caustic alkalies, ammonia and alkaline carbonates precipitate the base from its salts. Excess of caustic alkali redissolves heroine. When

boiled with water or dilute acids, but especially easily with sodium hydroxide solution, heroine readily undergoes hydrolysis into morphine and acetic acid:

$$C_{17}H_{17}ON(O.CO.CH_3)_2 + 2H_2O = C_{17}H_{17}ON(OH)_2 + 2C_2H_4O_2$$

In consequence of this behavior a part of the heroine at least will be recovered as morphine in an examination following the Stas-Otto procedure. Therefore, if it is essential that heroine be detected as such, the material to be examined should be made faintly alkaline with sodium carbonate and extracted at once with ether, better with chloroform, which dissolves free heroine base.

Detection

Unlike morphine, heroine gives no blue color with ferric chloride solution. In making this test, use an aqueous solution of neutral heroine hydrochloride. Excess of acid should not be present. Heroine does not reduce iodic acid solution. Moreover in distinction from morphine heroine does not immediately color blue a mixture of potassium ferricyanide and ferric chloride solutions. A blue color appears only after long standing, when heroine has undergone partial hydrolysis. On the other hand, heroine gives all the morphine tests in which saponification can take place as is the case in all reactions involving concentrated sulphuric acid. For this reason heroine responds to the morphine tests with the reagents of Froehde, Husemann, Marquis, Mecke and Pellagri.

According to Zernik, the following two tests may be used to differentiate heroine from morphine:

(a) A trace of heroine rubbed with a few drops of concentrated nitric acid (sp. gr. 1.4 = 60 per cent.) dissolves with yellow color. Extending from the centre of the drop to the margin, a green-blue color soon develops at ordinary temperature, but at once upon warming, and in time fades again to yellow.

(b) **Detection of Acetyl Groups.**—If a little alcohol is added to a trace of heroine, that has been heated with sulphuric acid, and the mixture is warmed, the odor of acetic ether may be detected. Obviously a positive result with this test is proof of the presence of heroine only in conjunction with the above morphine tests.

Heroine is considerably more toxic than morphine and, inasmuch as medicinal doses 0.003–0.91 gram are very close to the toxic dose, heroine has repeatedly been the cause of poisonings with fatal termination.

OXYDIMORPHINE

Oxydimorphine, or pseudo-morphine, $C_{34}H_{38}N_2O_6$, occurs in opium in small quantity and deserves attention since it appears in urine probably as a decomposition product of morphine in the human organism following administration of morphine. Dragendorff and other of the older authors assume "that most of the morphine is changed into inactive oxydimorphine or pseudo-morphine." The above assumption is easily explained in view of the fact that morphine, especially in alkaline solution, is converted into oxydimorphine by atmospheric oxygen and most of the oxidizing agents,

such as potassium permanganate, ammoniacal copper solution, potassium ferricyanide solution and other compounds of similar nature. But at the present time the author does not know of a case where pure morphine-free oxydimorphine has been obtained from the urine of persons poisoned by morphine or from that of morphine addicts.

$$\underset{\text{Morphine}}{2C_{17}H_{19}NO_3} + O = H_2O + \underset{\text{Oxydimorphine}}{C_{34}H_{38}N_2O_6}$$

Oxydimorphine is not toxic. Nascent hydrogen does not reconvert it into morphine. Oxydimorphine forms a white, crystalline powder almost insoluble in water, alcohol, ether and chloroform. Caustic alkalies precipitate it from solutions of its salts as a fine white powder soluble in excess of precipitant. Ammonia also liberates the base but redissolves it only when present in great excess. Strongly ammoniacal isobutyl alcohol, according to R. Kobert, is well suited for the extraction of oxydimorphine.

Detection

Oxydimorphine in most of its reactions shows a very strong resemblance to morphine, for example, in its behavior toward ferric chloride, iodic acid, concentrated nitric acid, and Froehde's reagent. Gadamer points out the following differences between the two alkaloids:

With Marquis' reagent oxydimorphine gives an intense yellow-red color; morphine a violet-blue color.

With concentrated sulphuric acid and cane-sugar oxydimorphine gives a blue color that gradually changes to green; morphine a red color.

With 8 drops of concentrated sulphuric acid 1 mg. of oxydimorphine gives a yellow solution; morphine a colorless solution. Upon gentle warming an intense green color appears; morphine gives a reddish color. After cooling, addition of 10 drops of water produces a rose color. If the liquid is washed into a test-tube with 10 drops of water and about 40 drops of water are added until the color disappears, the liquid becomes turbid from separation of oxydimorphine sulphate. If the liquid is now divided into 3 parts and 10–20 drops of concentrated nitric acid are carefully added to one part, 1 drop of sodium nitrite solution (5 per cent.) to the second part, and 1 drop of sodium hypochlorite solution to the third part, a dark violet color will appear in each solution, provided oxydimorphine is present. Morphine gives a red color.

Furthermore a trace of oxydimorphine and 2 drops of sodium hypochlorite solution rubbed together upon a watch-glass instantaneously produce a saffron-yellow color quickly changing to pale yellow. This mixture, if then stirred with 8 drops of concentrated sulphuric acid, takes on a fine emerald-green color. Morphine undergoes only a slight change, whereas codeine gives a sky-blue color.

PAPAVERINE

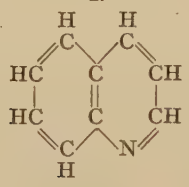
Papaverine, $C_{20}H_{21}NO_4$, constitutes about 0.5–1 per cent. of opium. When crude it is usually mixed with narcotine. To remove the latter, prepare the acid oxalate of papaverine which dissolves with difficulty in water. Crystallize this salt from boiling water until it dissolves in concentrated sulphuric acid without color. Convert papaverine oxalate into the hydrochloride by treatment with calcium chloride and then liberate the alkaloid with ammonia. This product crystallized from alcohol is pure papaverine.

Papaverine crystallizes in fine colorless prisms melting at 147°. This alkaloid is insoluble in water; soluble with difficulty in ether (1:250), cold alcohol and benzene; but freely soluble in hot alcohol, acetone and chloroform. These solutions are neutral, not bitter, and optically inactive. Papaverine is a weak base which dissolves in but does not neutralize acetic acid. Ether partially extracts it from an aqueous tartaric acid solution and completely extracts it from alkaline solution. Consequently this alkaloid appears in the Stas-Otto process in ether extract B. Chloroform extracts papaverine with almost as much ease from acid as from alkaline solution.

Constitution.—Papaverine is a monacid, tertiary base that combines with 1 molecule of an alkyl iodide to form crystalline addition products. As it forms no acetyl derivative with acetic anhydride, free hydroxyl is not present. But there are probably four methoxyl groups, for it loses four methyl groups when treated with hydriodic acid according to Zeisel's method. Consequently all oxygen atoms in papaverine are present as methoxyl groups. The researches of Goldschmiedt, extending from 1883 to 1898, have completely explained the constitution of papaverine. Moderate oxidation with potassium permanganate and sulphuric acid gives papaveraldine, $C_{20}H_{19}NO_6$, without breaking the carbon chain. Fusion with potassium hydroxide breaks the latter into nitrogen-free veratric acid and the nitrogenous base dimethoxy-isoquinoline:[1]

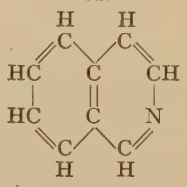
[1] Isoquinoline (II) is isomeric with quinoline (I) and like the latter is a monacid, tertiary base:

I.

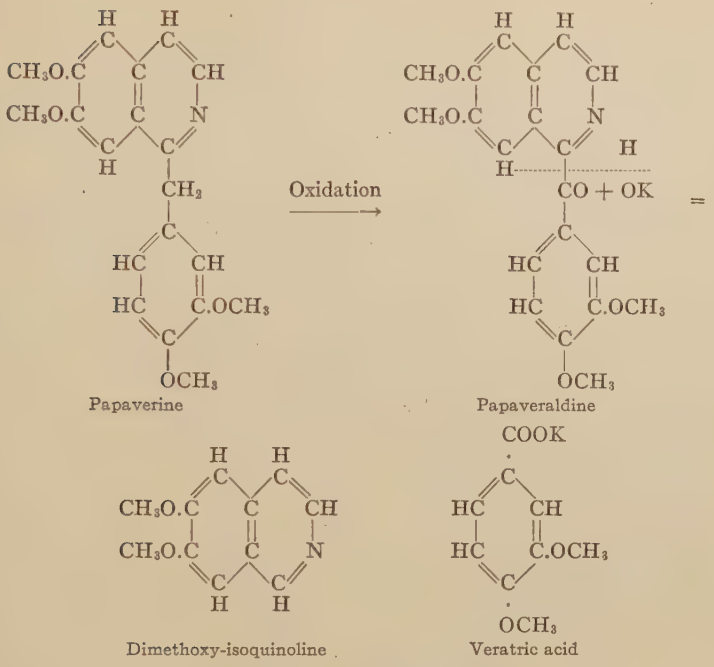


Quinoline

II.



Isoquinoline



Detection of Papaverine

The following general reagents precipitate papaverine in a dilution of 1:10,000: iodo-potassium iodide, phospho-molybdic acid and potassium bismuthous iodide; and in a dilution of 1:5000 tannic acid, gold chloride and potassium mercuric iodide give precipitates.

Special Reactions

1. **Concentrated Sulphuric Acid.**—A cold colorless solution of papaverine in this acid becomes dark violet upon gentle warming. But even a cold solution of impure papaverine in this acid is violet.

2. **Froehde's Test.**—Pure papaverine dissolves in this reagent without color. On the other hand, the commercial product gives a violet color gradually changing to blue-green, green and yellow.

3. **L. E. Warren's Test.**—Crush a very small crystal of potassium permanganate with a glass rod and intimately mix about 0.0005 gram of papaverine with the powder. Stir this mixture into about 0.2 cc. of Marquis' reagent. A green color, almost instantly changing to blue, appears. The latter color deepens into an intense violet-

blue which after some time becomes bluish green, green and finally dirty brown.

Of thirty-nine alkaloids tested, the only one in any way simulating papaverine was an unnamed alkaloid separated from sanguinaria.

4. **Concentrated Nitric Acid.**—This acid as well as Erdmann's reagent dissolves pure papaverine without color. Heat to boiling a solution of 1 part of papaverine with 10 parts of nitric acid (sp. gr. 1.06 = 10 per cent. HNO_3). As the solution cools, yellow crystals of the nitrate of nitro-papaverine, $C_{20}H_{20}(NO_2)NO_4.HNO_3.H_2O$, appear. Pale yellow prisms of nitro-papaverine, $C_{20}H_{20}(NO_2)$-$NO_4.H_2O$, may be obtained from this nitrate by means of ammonia.

5. **Mecke's Test.**—This reagent dissolves pure papaverine in the cold with a greenish color which soon becomes dark steel-blue and finally deep violet. Upon warming the solution a dark violet color appears at once.

Physiological Action.—According to Pal, papaverine lowers the tonus of smooth muscles but especially when increased through illness. Papaverine hydrochloride, used internally or subcutaneously in doses of 0.03–0.08 gram, or intravenously in doses from 0.01–0.02 gram, has proved a harmless and frequently a very active agent in the treatment of many vascular spasms. Frequently in doses of 0.005–0.05 gram several times daily it has proved quite useful in treating diarrhoea in children. It exerts a relaxing action upon the uterus. Its physiological action is similar to that of narcotine. In small doses papaverine has a narcotic action upon mammals, in larger doses it produces tremors, muscular tension and convulsions. In man after 0.18 gram of papaverine great languor and muscular weakness have been observed (Lewin). Following internal or subcutaneous administration, papaverine has been found in almost all organs, for example, the liver as well as in bile and urine.

THEBAINE

Thebaine, $C_{19}H_{21}NO_3$ or $C_{17}H_{15}(OCH_3)_2NO$, occurs in opium to the extent of 0.2–0.5 per cent. Crude commercial thebaine may be purified by solution in a little dilute acetic acid, decolorizing by means of animal charcoal, and then adding powdered tartaric acid. After long standing thebaine tartrate separates and is recrystallized from a little boiling water. Free thebaine is again precipitated by ammonia and finally recrystallized from hot alcohol. Thebaine may also be separated by means of sodium salicylate.

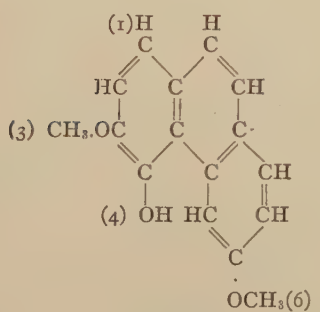
From hot dilute alcohol thebaine crystallizes in colorless leaflets resembling benzoic acid; from strong alcohol in thick prisms. It

melts at 193°. In cold water it is almost insoluble but easily soluble in alcohol, benzene and chloroform. It requires 140 parts of ether for solution. Solutions of thebaine are laevo-rotatory and have an alkaline reaction. Its taste is not bitter. Caustic alkalies, ammonia, milk of lime, sodium carbonate and bicarbonate precipitate the free base from solutions of its salts. The hydrochloride, salicylate and acid tartrate are crystalline compounds. Thebaine has scarcely any narcotic action and may be considered as a pure convulsive poison (von Schroeder).

Constitution.—Thebaine is a rather strong, monacid, tertiary base, forming as a rule well crystallized salts with acids. But excess of acid, especially mineral acid, usually decomposes these salts with ease. Being a tertiary base, it easily combines with methyl iodide forming thebaine iodomethylate, $C_{15}H_{21}NO_3.CH_3I$, crystallizing in prisms. Two of the three oxygen atoms in thebaine are methoxyl-groups ($-OCH_3$) and the third probably forms an ether-like combination, a so-called bridge-oxygen. The thebaine molecule appears not to contain hydroxyl. Therefore thebaine differs from morphine in not being soluble in sodium or potassium hydroxide solution.

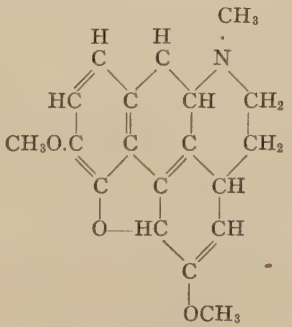
Heated with acetic anhydride, thebaine gives the acetyl derivative of the phenol thebaol, $C_{16}H_{14}O_3$, and a nitrogenous product, methyl-oxy-ethylamine, $CH_3.NH.CH_2.CH_2.OH$. R. Pschorr has synthesized thebaol, or the methyl ether of thebaol, and shown by this synthesis that thebaol is 3, 6-dimethoxy-4-oxy-phenanthrene (I). Pschorr assigns to thebaine the structural formula (II) which is analogous to that of apomorphine and morphine (see pages 220 and 227):

I.



Thebaol

II.



Thebaine (R. Pschorr)

Detection of Thebaine

Ether and chloroform extract thebaine from alkaline aqueous solution and consequently this alkaloid appears in ether extract B, if the Stas-Otto procedure is followed. The general reagents,

phospho-tungstic acid, iodo-potassium iodide, potassium mercuric iodide and potassium bismuthous iodide precipitate thebaine even from very dilute solutions. Thebaine gives the following color reactions:

1. **Concentrated Sulphuric Acid.**—Thebaine dissolves in this acid with a deep red to blood-red color that gradually becomes yellowish red. The reagents of Erdmann, Froehde and Mandelin give the same result.

2. **Concentrated Nitric Acid.**—Thebaine dissolves in this acid with a yellow color.

3. **Mecke's Test.**—In the cold thebaine dissolves in this reagent with a deep orange-red color that soon fades and with heat becomes dark brown.

4. **Marquis' Test.**—Thebaine dissolves in this reagent with a yellow-red to brown color.

THEOBROMINE

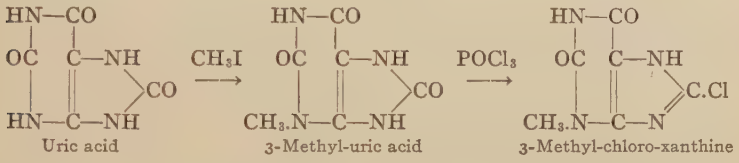
Theobromine or 3,7-dimethyl-xanthine, $C_5H_2N_4O_2(CH_3)_2$, is a constituent of cacao beans, occuring in the cotyledons to the extent of 1.4–1.8 per cent., whereas the hulls contain only 0.5–1.3 per cent. Young, air-dried cacao leaves also contain as high as 0.55 per cent. of theobromine, whereas old leaves contain almost none. Cola nuts and guarana paste contain only small quantities of theobromine together with a relatively large quantity of caffeine.

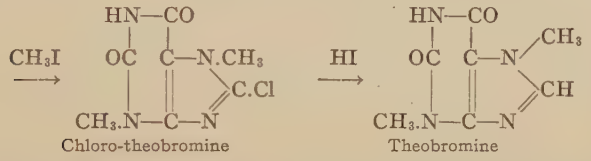
Properties.—Theobromine is a bitter, white powder consisting of microscopic needles. It sublimes almost without decomposition when carefuly heated! One part of theobromine dissolves in 3280 parts of water at 18° and in 149 parts at 100°; in 1500 parts of 90 per cent. alcohol at 15°; in 3845 parts of acetic ether and 105 parts of boiling chloroform. It dissolves in 4284 parts of cold absolute alcohol and in 422 parts of boiling alcohol. It is almost insoluble in pure ether but easily soluble in phenol. Solutions of theobromine react neutral and are optically inactive. Theobromine manifests the properties of a weak base and at the same time those of a weak acid. Consequently it dissolves easily in sodium or potassium hydroxide solution, as well as in mineral acids, forming salts. Although salts of theobromine with acids crystallize, they are partially decomposed by water into theobromine and acid and also when heated to 100°, provided the acid is volatile. Theobromine hydrochloride, $C_7H_8N_4O_2.HCl.H_2O$, obtained by dissolving theobromine in hot concentrated hydrochloric acid, forms colorless needles. Of the salts formed with bases, that containing silver, $C_7H_7N_4O_2Ag.1.5H_2O$, deserves mention. It appears as a white crystalline powder almost insoluble in water, when a solution of theobromine in dilute ammonia is treated with silver nitrate and then boiled for some time.

Synthetic Theobromine.—E. Fischer obtained theobromine by heating lead xanthine for several hours with methyl iodide at 100°:

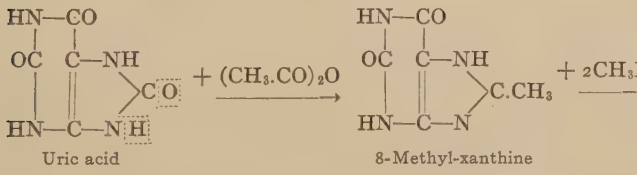
$$C_5H_2O_2N_4Pb + 2CH_3I = C_5H_2O_2N_4(CH_3)_2 + PbI_2$$

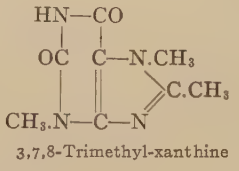
Upon the basis of this synthesis, theobromine is regarded as dimethyl-xanthine. E. Fischer also obtained theobromine by starting from uric acid. By means of methyl iodide at about 80° uric acid in alkaline solution was converted into 3-methyl-chloro-xanthine. The latter when methylated gave chloro-theobromine which was reduced by nascent hydrogen, that is, by hydriodic acid, to theobromine:

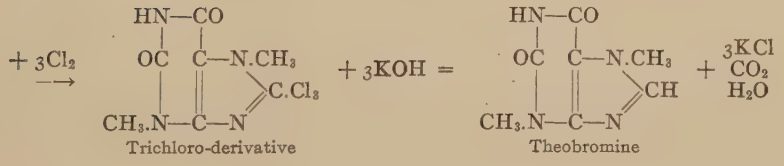




Technical Preparation.—Uric acid heated with acetic anhydride gives 8-methyl-xanthine. This compound in the form of its di-potassium salt heated with methyl iodide is converted into 3,7,8-trimethyl-xanthine. The vigorous action of chlorine upon the latter gives a trichloro-derivative from which through cleavage of carbon dioxide, water and potassium chloride by means of potassium hydroxide solution theobromine is obtained:







Physiological Properties.—Theobromine gives rise to nearly the same physiological effects given by the other purine derivatives of this series, caffeine and theophylline (see caffeine, page 147). It is an excellent diuretic. According to Schmiedeberg, however, in producing muscular rigidity its action is stronger than that of caffeine but it does not act as strongly as the latter in causing convulsions. Veley and Waller[1] allowed 0.02–0.003 n-caffeine and theobromine solutions in sodium chloride to act upon isolated muscle and determined how long a time elapsed before the muscle was not irritated. In these experiments the toxicity of theobromine to that of caffeine was in the ratio of 1.7:1. According to Kobert, theobromine exerts a specific irritating action upon the kidneys. But very likely the toxicity of theobromine is less than that of theophylline. Not until the daily dose of theobromine exceeds 2–5 grams is it said to cause vomiting, violent headache and albuminuria.

Detection of Theobromine

Amalic Acid Test.—If theobromine is quickly evaporated to dryness upon the water-bath, with about 100 times the quantity of chlorine water, or a solution of theobromine in dilute hydrochloric acid with some potassium chlorate, a red to red-brown residue remains. The latter brought in contact with a little ammonia gives a purple-violet color. This test may be made by covering the dish containing the residue with a glass plate previously moistened with a drop of strong ammonia. More explicit details concerning this test will be found under caffeine upon page 148.

Chloroform is the best solvent for extracting theobromine from an acidified solution. Gadamer gives the following directions for detecting theobromine. Dry the material thoroughly upon the water-bath or in a drying-closet, free it from fat by means of petroleum ether, and then extract under reflux with alcohol (80 per cent. by volume) faintly acidified with sulphuric acid. Remove alcohol from the filtered extracts by distillation or evaporation, neutralize the residue with magnesium oxide, dry and extract for a long time in a Soxhlet with chloroform. For purification stir the residue remaining after evaporation of chloroform with water containing sulphuric acid, then filter and again extract theobromine with hot chloroform. The solvent now leaves the purine base in a nearly pure condition. The residue may finally be subjected to sublimation between two watch-glasses. The distinctly crystalline sublimate deposited upon the upper watch-glass, in case theobromine is present, may be used for the amalic acid test.

[1] V. H. Veley and A. D. Waller: Relative Toxicity of Theobromine and Caffeine, as expressed in the Direct Action upon the Contractility of Isolated Muscle. Proc. Royal Soc. London. Series B. 82 (1910), 568.

Urine.—Theobromine like caffeine is also partially demethylated in the human organism so that urine contains 3- and 7-methyl-xanthine separated in part as such. Stomach-contents, vomitus, blood and urine are suitable material for examination. It is a notable fact that the three xanthine bases, caffeine, theopylline and theobromine, which find use in medicine as diuretics, are quite stable toward putrefaction.

Diuretine or Sodium-Theobromine-Sodium Salicylate

Theobromine dissolves in water with great difficulty. For this reason it is convenient to use the double compound diuretine which has an alkaline reaction and is soluble in water. Diuretine as well as the analogous agurine, theobromine-sodium acetate, are much more prone than caffeine to give rise to gastric and intestinal disturbances (H. Meyer-Gottlieb).

Preparation.—Dissolve theobromine in the calculated quantity of sodium hydroxide, adding the equivalent amount of sodium salicylate and evaporating to dryness, best *in vacuo*. Diuretine corresponding to the formula, $C_7H_7O_2N_4$-$Na.C_6H_4(OH)COONa$, is a white, inodorous powder having a sweetish saline and somewhat alkaline taste. Its solutions in water have an alkaline reaction. It contains about 45 per cent. of theobromine.

Estimation of Theobromine in Diuretine

Dissolve 2 grams of diuretine in 10 cc. of water, adding sufficient n-hydrochloric acid to produce a faint red color upon blue litmus paper. Then add a drop of ammonia, stir occasionally and allow the mixture to stand for 3 hours at ordinary temperature. Collect the theobromine that separates upon a weighed filter, or better in a weighed Gooch crucible, rinse twice using 10 cc. of cold water each time, dry at 100° and weigh. The content of theobromine in 2 grams of diuretine should be at least 0.8 gram.

Notes.—The quantity of hydrochloric acid added to the diuretine solution should not be more than is necessary to decompose sodium-theobromine:

$$C_7H_7O_2N_4Na.C_6H_4(OH)COONa + HCl = C_7H_8O_2N_4 + NaCl + C_6H_4(OH)\text{-}COONa$$

Excess of hydrochloric acid would also decompose sodium salicylate causing separation of salicylic acid and in addition a small quantity of theobromine would be dissolved.

THEOPHYLLINE

Theophylline, theocine or 1,3-dimethyl-xanthine, $C_7H_8O_2N_4.H_2O$ or $C_5H_2O_2N_4(CH_3)_2.H_2O$, was first obtained by Kossel from the residues left after separation of caffeine from tea. Details of the synthesis of theophylline from malonic acid and dimethyl-urea are given under caffeine (see page 147). In that place the structural formula of theophylline is also discussed.

Theophylline forms thin monoclinic plates or needles melting at 264–265°. It dissolves easily in hot water and boiling alcohol but with difficulty in cold water and cold alcohol. Theophylline salts usually crystallize well. In accordance with its double character of base and acid, theophylline forms salt-like compounds both with acids and strong bases. Consequently this purine derivative is soluble in caustic alkalies as well as in acids.

Sodium-theophylline, $C_7H_7O_2N_4Na.H_2O$, is a white crystalline powder soluble in water with strong alkaline reaction. It forms double salts corresponding to diuretine, for example, sodium-theophylline-sodium acetate, $C_7H_7O_2N_4Na.C_2H_3O_2Na$, a white powder having an alkaline reaction.

Physiological Properties.—Theophylline exerts strong diuretic action and is very frequently used especially in dropsy. In cases where other diuretics have failed, frequently theophylline has given excellent results. But these results have often been attended with unpleasant secondary effects, for theophylline sometimes causes nausea, vomiting, epileptic convulsions, haemorrhages of the gastric mucosa and gastric ulcers. Experiments upon animals have shown that irritation of the kidneys is produced. As the maximal daily dose, 0.8 gram of pure theophylline is recommended.

Detection of Theophylline

Theophylline may be extracted from material by the same method given for theobromine. It may also be extracted by chloroform from an acidified solution and may be sublimed, best between two watch-glasses. The sublimate formed upon the upper watch-glass may be used for the amalic acid test described for theobromine (see page 414). Theophylline differs from caffeine and theobromine in that its solution in sodium hydroxide gives a red color with the diazo reagent.[1]

TOXALBUMINS

Toxalbumins are toxic, protein-like substances that are either already formed in the plant or animal organism, or are produced in the metabolism of pathogenic micro-organisms. As yet these substances have not been isolated in a state of purity as individual chemical compounds. As a matter of fact, the chemical and physiological properties of such vegetable toxalbumins as abrin, ricin, robin and crotin are given by substances obtained from some particular part of the plant by a definite method. The vegetable toxalbumins

[1] See "Preparation of Reagents," page 641.

mentioned possess the common property of clumping, agglutinating and precipitating red blood-corpuscles. Therefore R. Kobert classifies them as "vegetable agglutinines." A trace of one of these agglutinines, added to defibrinated blood in a test-tube, causes clumping into a mass resembling sealing-wax. Abrin, ricin and crotin also cause coagulation of milk.

ABRIN

This toxalbumin occurs in jequirity seeds from Abrus precatorius. Remove the seed envelopes and extract the finely divided seeds with 4 per cent. sodium chloride solution. Concentrate the filtered liquid *in vacuo* and acidify with acetic acid. Precipitate abrin from this solution by addition of sodium chloride and finally purify by dialysis. Abrin is an amorphous, highly toxic powder not entirely free from ash. Though abrin and ricin are alike in some respects, they are not identical.

RICIN

This intensely toxic toxalbumin constitutes 2.8–3 per cent. of castor bean. Remove the seed envelopes and subject the seeds to powerful pressure to remove as much oil as possible. Then extract with 10 per cent. sodium chloride solution. Saturate the filtered extract at the same time with magnesium and sodium sulphate and keep for some time in the cold at room temperature. Place the precipitate containing ricin, in a parchment paper dialyzing tube and dialyze for several days. Finally dry the residual ricin *in vacuo* over sulphuric acid.

Ricin is an amorphous, highly toxic powder containing ash and easily soluble in 10 per cent. sodium chloride solution. This toxalbumin, dissolved in sodium chloride solution, gives the protein reactions. Ricin possesses in high degree the power of agglutinating blood-corpuscles, that is, the blood-corpuscles clump together leaving a clear serum. For this test-tube experiment use defibrinated blood, not diluted blood, nor blood mixed with physiological salt solution, that is, 0.9 per cent. solution. Ricin, according to Elfstrand, agglutinates red blood-corpuscles of the guinea-pig even in dilution of 1:600,000. Ricin agglutinates the blood of all mammals but not to the same degree. Removal of serum from blood and substitution of physiological salt solution strengthen rather than weaken the agglutinating action of ricin. The inference is that serum must

possess a certain anti-agglutinating action. Separation of red blood-corpuscles into stroma and haemoglobin[1] shows that ricin has not changed haemoglobin in the least. But the stromata in the same manner as the blood-corpuscles have been altered.

To detect ricin in castor bean press-cake, or in feeds containing castor beans, extract the finely divided material with physiological salt solution at room temperature, filter and make the agglutination test in a test-tube with undiluted, defibrinated blood and with blood diluted with physiological salt solution.

CROTIN

Crotin is a substance obtained from the seeds of Croton Tiglium. Remove the seed envelopes, express the oil and treat as described for abrin and ricin. Crotin chemically is quite similar to ricin. Abrin and ricin agglutinate blood-corpuscles of all warm-blooded animals thus far tested but crotin does not manifest the same behavior with all kinds of blood. (See R. Kobert's "Intoxikationen.")

Coagulation of Blood and Defibrinated Blood

Blood is a transparent fluid, the blood-plasma, suspended in which is a very large number of solid particles, red and white blood-corpuscles. Outside the organism blood coagulates even in a few minutes after being drawn. In the clotting of blood a very difficultly soluble protein called fibrin separates. If the blood is still, the clot is a solid mass that gradually contracts and exudes a clear liquid, usually yellow, the blood-serum. The coagulum, thus formed and enveloping the blood-corpuscles, is called the crassamentum (Placenta sanguinis). But if the blood is whipped during coagulation, fibrin separates in threads. The fluid separated from the latter is defibrinated blood consisting of blood-corpuscles and blood-serum. To obtain defibrinated blood, whip fresh blood removed from a vein with twigs and fibrin will separate upon the latter. Or run fresh blood into an Erlenmeyer flask containing iron filings and shake vigorously for several minutes. Fibrin is precipitated on the filings.

Coagulation of blood may be retarded by cooling suddenly to low temperature. By drawing the blood direct from the vein into a neutral salt solution and stirring, for example, magnesium sulphate solution (1 volume of salt solution +3 volumes of blood), a blood-salt mixture that will remain uncoagulated for a day is obtained. Blood may be added to sufficient dilute potassium oxalate solution to give a mixture containing 0.1 per cent. of oxalate. Soluble calcium salts of the blood are precipitated by oxalate and blood loses its power of coagulating. To prepare a non-coagulating blood-plasma, pour blood into sodium fluoride

[1] Blood-corpuscles are made up of two principal components, namely, the stroma, which constitutes the true protoplasm, and the intraglobular contents, the chief constituent of which is haemoglobin.

solution until it contains 0.3 per cent. of NaF. Aqueous solution of sodium citrate also retards coagulation of blood.

CYTISINE

Cytisine, $C_{11}H_{14}N_2O$, occurs in ripe seeds of Golden chain (Cytisus Laburnum) to the extent of about 1.5 per cent. Cytisine and the alkaloid originally called ulexine, isolated from seeds of Ulex europaeus, are identical (A. Partheil).

Cytisine crystallizes in large, colorless, inodorous prisms melting at 152° and subliming at higher temperature if carefully heated. It dissolves freely in water, alcohol, chloroform and acetic ether; less easily in commercial ether, benzene and acetone; and is almost insoluble in petroleum ether and absolute ether.

Cytisine is a strong secondary base and very toxic. Although capable of combining with 1 or 2 molecules of hydrochloric acid, this compound behaves in other respects like a monacid base. Only salts containing one equivalent of acid crystallize well. Nitrous acid converts this secondary base into nitroso-cytisine, $C_{11}H_{13}ON_2$-NO, crystallizing in needles. Nitrous fumes appear, if cytisine is warmed upon the water-bath with twice the amount of concentrated nitric acid, and the solution at once becomes reddish yellow to brown. This solution poured into water gives a precipitate of nitro-nitroso-cytisine, $C_{11}H_{12}ON(NO_2)$N-NO. This compound crystallizes from water in pale yellow scales melting at 242–244°. According to van de Moer[1] cytisine is an apopilocarpine.

Toxic Action.—Cytisine produces convulsions, its action in this respect being quite similar to that of strychnine. But unlike the latter alkaloid it also irritates the gastro-intestinal mucosa even causing bloody inflammation. Cytisine also differs from strychnine in stimulating the vomiting center. Consequently after doses of cytisine or laburnum preparations, human beings and animals capable of emesis thus rid the organism of a large part of the poison. Like strychnine, cytisine stimulates the respiratory and vaso-motor centers. Finally, as in strychnine intoxication, death results from paralysis of these two centers. A part of the cytisine leaves the organism unchanged and appears in the urine.

Detection of Cytisine

Prepare an aqueous tartaric acid solution of stomach-contents, vomitus or parts of organs, following the general procedure for alkaloids. To remove final traces of fatty acids and fat, shake this solution well with ether. Withdraw the aqueous solution, make alkaline with sodium hydroxide solution, and extract thoroughly with chloroform or better with isobutyl alcohol. Evaporate the chloroform or isobutyl alcohol extracts and test the residue as follows for cytisine:

1. Van de Moer's[2] Test.—Ferric chloride solution colors cytisine and its salts blood-red. Dilution with water, or acidification, discharges this color. Hydrogen peroxide also produces the same result. The solution containing hydrogen peroxide, warmed upon the water-bath, becomes intensely blue.

[1] J. van de Moer: Synthesis of Cytisine. Nederl. Tijdschr. Pharm. 7 (1895), 362.

[2] Ber. d. Deutsch. Pharm. Ges. 5 (1895), 267.

2. **Rauwerda's**[1] **Test.**—A little nitrobenzene, containing dinitro-thiophene, poured upon cytisine gives a fairly stable, red-violet color. A similar color given by coniine is very unstable.

3. **Nitro-Nitroso-Cytisine Test.**—Nitro-nitroso-cytisine (see above), formed by concentrated nitric acid, serves to detect small quantities of this alkaloid. Nitro-nitroso-cytisine crystallizes from 94 per cent. alcohol in compact prisms and from 50 per cent. alcohol in flat plates. Water again precipitates it unchanged from solution in concentrated hydrochloric acid.

GLUCOSIDES AND SAPONIN SUBSTANCES

DIGITALIS GLUCOSIDES

The digitalis plant, Digitalis purpurea L., contains in all its parts, but especially in leaves and seeds, medicinally useful substances belonging to the glucoside group. Thus far three digitalis glucosides have been isolated as well characterized crystalline compounds of homogeneous composition. These are digitalin in the stricter sense, or Digitalinum verum crystallisatum Kiliani, $C_{35}H_{56}O_{14}$; digitoxin, $C_{44}H_{70}O_{14}$; and digitonin, $C_{55}H_{90}O_{29}$. A fourth glucoside called digitalein seems as yet not to have been obtained wholly pure.

DIGITOXIN

Digitoxin, $C_{44}H_{70}O_{14}$, occurs almost exclusively in digitalis leaves. This very active and highly toxic compound is almost entirely insoluble in water and ether but soluble in alcohol and chloroform. Consequently ether precipitates it from chloroform solution. Warmed with concentrated hydrochloric acid, digitoxin gives a green to brownish green color. According to Kiliani, it crystallizes from 85 per cent. alcohol in leaflets melting at 145°.

Pure digitoxin, according to the recent exhaustive investigation of Cloetta,[2] melts considerably higher, that is, sharply at 252–253°. It is obtained by dissolving the impure product in hot chloroform, adding ether to the solution while warm, and quickly filtering off the crystals before the solution has cooled. Once impurities have been removed, the melting-point 252° does not change, no matter whether this pure digitoxin is finally crystallized from alcohol or acetone with addition of water, or from chloroform with addition of

[1] A. Rauwerda: Contributions to the Knowledge of Cytisine and Its Alkyl-derivatives. Chem. Zentralbl. 1900 II. 268; Nederl. Tijdschr. Pharm. 12 (1900), 161.

[2] M. Cloetta: Contribution to the Knowledge and Pharmacology of Digitoxin and Its Cleavage-products. Arch. f. exper. Path. u. Pharm. 88 (1920), 113.

ether, or whether some alkali is added to the solvent. According to Cloetta, pure digitoxin is prepared from the crude product by dissolving the latter in warm alcohol and adding warm water to this solution until the alcohol-content is about 40 per cent. As the solution cools, digitoxin separates in glistening crystals that appear after several recrystallizations as beautiful plates. This plate-form is characteristic of this manner of crystallizing digitoxin. It is always obtained in the same microscopic form when the product is pure.

Pure digitoxin is soluble in alcohol, acetone, chloroform, glacial acetic acid, soluble with great difficulty in ether, and insoluble in water. From solution in alcohol and chloroform it is precipitated by ether in beautiful crystalline plates. Keller's reaction is characteristic of digitoxin. Elementary analysis gives digitoxin the formula $C_{41}H_{70}O_{14}$. A solution of 0.4 mg. of digitoxin in warm dilute alcohol injected into a lively frog weighing about 35 grams will cause stasis of the heart in 12–15 minutes.

Cloetta has given the following brief summary of the results of his exhaustive investigations:

Digitoxin heated in high vacuum yields a compound that sublimes and melts at 114°. It is soluble in water, alcohol, ether, chloroform, acetone, glacial acetic acid, and crystallizes very well. The blue-green color given to glacial acetic acid in Keller's test is even more intense than that given by pure digitoxin. This volatile substance gives none of the sugar reactions, adds bromine and iodine, has the elementary formula $C_8H_{14}O_4$, and is pharmacologically inactive. Cloetta designates the residual substance, remaining after this volatile product has separated from digitoxin, as digitan having the formula $C_{36}H_{56}O_{10}$. Its pharmacological behavior is like that of digitoxin. Acids break up digitan into 1 molecule of digitoxigenin and 2 molecules of digitoxose.

The result of acid cleavage of pure digitoxin is the quantitative formation of 1 molecule of digitoxigenin, 2 molecules of digitoxose, and 1 molecule of an oily substance. The latter is the equivalent of the substance that sublimes in crystals. A close relationship exists between the two products. The crystals treated with acid yield a substance apparently identical with the oil. This oil gives the digitoxose reaction strongly, is not a sugar, and is pharmacologically inactive. A characteristic of digitoxigenin, obtained from digitoxin by acid cleavage, is that it first gives a yellow ring in Kel-

ler's reaction and then colors the glacial acetic acid light green. Agitation produces a stable, fluorescent, emerald-green color, whereas that given by impure digitoxigenin is reddish. Pure digitoxigenin melts at 245° and has the elementary formula $C_{24}H_{36}O_4$. It still has a distinct action upon the heart but this differs somewhat qualitatively and quantitatively from that given by digitoxin. Yet at the same time digitoxigenin is a powerful central convulsive poison.

Digitoxigenin warmed with acid yields anhydro-digitoxigenin, $C_{24}H_{34}O_3$, melting at 184°. It may be obtained direct from digitoxin after more intense acid cleavage. These cleavages proceed quantitatively. Anhydro-digitoxigenin, though no longer active, possibly still causes convulsive action.

The action of digitoxin upon the heart is to be ascribed to the presence of several hydroxyl-groups. Digitoxin treated with benzoyl chloride or stearyl chloride yields crystalline substitution products into the formation of which five acid radicals always enter. These derivatives are pharmacologically inert. When only two hydroxyl-groups are closed, the action of the product upon the heart is weakened.

Benzoylation of digitan also reduces the action of this substance upon the heart.

Acetylation of digitoxigenin introduces only one acetyl-group. This acetyl derivative no longer exerts an action upon the heart but possibly still retains convulsive action. In this respect its behavior is similar to that of digitoxigenin. Consequently in both preparations the hydroxyl-group of digitoxigenin responsible for heart action has been put out of commission, whereas the cause of convulsive action apparently must be looked for in some other group.

The cleavages of digitoxin and digitan may be expressed in the following equations:

(a) Cleavage of digitoxin *in vacuo:*

$$\underset{\text{Digitoxin}}{C_{44}H_{70}O_{14}} = \underset{\text{Digitan}}{C_{36}H_{56}O_{10}} + \underset{\substack{\text{Volatile}\\\text{crystals}}}{C_8H_{14}O_4}$$

(b) Quantitative acid cleavage of digitan:

$$\underset{\text{Digitan}}{C_{36}H_{56}O_{10}} + 2H_2O = \underset{\text{Digitoxigenin}}{C_{24}H_{36}O_4} + 2\underset{\text{Digitoxose}}{C_6H_{12}O_4}$$

(c) Primary acid cleavage of digitoxin:

$$\underset{\text{Digitoxin}}{C_{44}H_{70}O_{14}} + 2H_2O = \underset{\text{Digitoxigenin}}{C_{24}H_{36}O_4} + 2\underset{\text{Digitoxose}}{C_6H_{12}O_4} + \underset{\text{Oil}}{C_8H_{14}O_4}\ (?)$$

In the last cleavage the elementary formula of the volatile crystals has been arbitrarily introduced for the oil, since it comes nearest to the actual relations.

Benzoyl-digitoxin.—By dropping benzoyl chloride with good agitation into a pyridine solution of digitoxin cooled at 0°, Cloetta obtained pentabenzoyl-digitoxin. Addition of petroleum ether to a benzene solution of this compound causes it to appear in rosette-shaped crystals made up of small rods. It is easily soluble in ether, acetic ether, benzene and chloroform and fairly easily in alcohol. At 145° it sinters and melts at 156–158°.

Keller's Digitoxin Reaction.—Dissolve 0.5–1 mg. of digitoxin in 3–4 cc. of glacial acetic acid containing a trace of iron and carefully add this glacial acetic acid solution to about the same quantity of concentrated sulphuric acid so that the two liquids do not mix. At the zone of contact the blue-green color of the glacial acetic acid characteristic of digitoxin develops upward.

Kiliani's Digitoxin Reaction.—Dissolve a trace of digitoxin in 3–4 cc. of glacial acetic acid containing iron (100 cc. of glacial acetic acid and 1 cc. of 5 per cent. ferric sulphate solution). Cautiously add sulphuric acid containing iron (100 cc. of sulphuric acid and 1 cc. of 5 per cent. ferric sulphate solution) in about the same quantity as an under layer. A dark zone appears where the two solutions meet, above which after a few minutes a blue band is visible. After some time the entire acetic acid layer becomes deep indigo-blue.

Lafon's[1] Procedure for Digitoxin Test.—In this procedure a well-cooled mixture of equal volumes of 95 per cent. alcohol and concentrated sulphuric acid is used together with a very dilute aqueous ferric chloride solution having a scarcely perceptible yellow color. Moisten a small crystal of digitoxin upon a watch-glass with a drop of alcoholic sulphuric acid and then add a drop of ferric chloride solution. An intense blue-green color quickly appears at the line of contact of the two drops. Only digitoxin gives this reaction.

DIGITALIN

Digitalin, $C_{35}H_{36}O_{12}$, occurs only in digitalis seeds (Kiliani). It is somewhat soluble in water (1:1000); easily soluble in alcohol and chloroform; but insoluble in ether. At 210° it sinters and melts at 217°. It tastes faintly bitter and is very active, that is, it is a heart-poison. Boiled in alcoholic solution with very dilute hydro-

[1] L. Garnier: Color Reactions of the Toxic Digitalis Glucosides. Journ. Pharm. et Chim. (6) 27 (1908), 369.

chloric acid, it undergoes hydrolysis into digitaligenin and the two sugars dextrose and digitalose:

$$\underset{\text{Digitalin}}{C_{35}H_{56}O_{14}H_2O} = \underset{\text{Digitaligenin}}{C_{22}H_{30}O_3} + \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Digitalose}}{C_7H_{14}O_5(?)}$$

Concentrated sulphuric acid colors pure digitalin orange-yellow. The color of this solution soon becomes blood-red and upon addition of a little bromine water cherry-red and blue-red. A drop of nitric acid or ferric chloride solution may be substituted for bromine water. This reaction is more certain and of longer duration (1–2 hours), if a trace of digitalin is merely dissolved direct in sulphuric acid, or in Kiliani's sulphuric acid containing iron.

Digitalin dissolved in concentrated hydrochloric acid produces a gold-yellow color becoming upon warming garnet-red to violet-red.

Many recommend the Grandeau and Trapp tests for the detection of digitalin. But these two reactions are not characteristic of digitalin! Consequently they cannot be used for the exclusive detection of digitalin, since with these two reagents many other plant substances give the same or very similar colors. Bing[1] recommends making these tests as follows:

1. Grandeau's Test.—Add 3 cc. of pure concentrated sulphuric acid to a test portion of substance in a small test-tube and then 3 drops of cold saturated aqueous bromine solution. This mixture when gently shaken will become rose-red to violet-red, if digitalin is present. Among other substances giving a similar color under the same conditions are agaricin, abietic acid, amygdalin, brucine, delphinine, convallamarin, camphor, salicin, solanine, veratrine, cyclamin, cytisine (Bing).

2. Trapp's Test.—Pour about 5 cc. of water upon the substance, add an equal volume of phospho-molybdic acid solution and heat the mixture in a boiling water-bath. A green color will appear if digitalin is present. If ammonium chloride is then added to the solution when cold, the green color will change to blue. The solution reheated in the water-bath will become colorless. Under the same conditions the same or similar colors are given by brucine, convallamarin, cyclamin, delphinine, helleborin, heroine, morphine, ricin and strychnine. Aniline hydrochloride and phenacetine also behave similarly. Consequently Trapp's reaction is not characteristic of digitalin!

Detection of Digitoxin in Organs and Urine

(Cloetta and Fischer[2])

1. In Organs.—Render the comminuted material faintly acid with acetic acid, extract with 50 per cent. alcohol, evaporate the

[1] C. Bing: Arch. internat. de pharm. et de thér. 12 (1906), 337; Pharm. Zentralbl. 45 (1906), 154.

[2] M. Cloetta and H. F. Fischer: Behavior of Digitoxin in the Organism Archiv. f. experim. Pathol. u. Pharmak. 54 (1906), 294.

filtered alcoholic extract to a syrup, and add alcohol to precipitate salts and dissolved protein substances. Filter, thoroughly extract the precipitate with alcohol, evaporate the extracts to dryness upon the water-bath, dissolve the residue in 10 per cent. alcohol, add a few drops of ammonia, and extract with chloroform. Evaporate the chloroform, dissolve the residue in glacial acetic acid, and use this solution for Keller's or Kiliani's digitoxin test. Or dissolve the residue in 3 cc. of chloroform, add 10 cc. of ether, 70 cc. of petroleum ether, and allow to stand well covered for 48 hours. Decant the solution from the precipitate that forms and examine the latter for digitoxin by the above tests.

2. **In Urine.**—Render the urine faintly alkaline with ammonia and evaporate to a syrup. Stir the residue well with about 5 parts of absolute alcohol and add an equal quantity of chloroform and benzene. After thoroughly shaking the resulting deep brown solution, add basic lead acetate until the supernatant liquid has only a pale yellow color. Filter and wash the precipitate with a mixture of 5 parts of alcohol, 1 part of chloroform and 1 part of benzene. Remove lead from the filtrate with hydrogen sulphide, filter from lead sulphide, and evaporate the filtrate to a thin syrup. Dissolve the latter in 10 per cent. alcohol, render alkaline with a little ammonia, and extract repeatedly with chloroform-benzene (3:1). Should these extracts still have a strong color, treat with pure clay (Bolus alba). Use the residue from evaporation for Keller's or Kiliani's digitoxin test. About 75 per cent. of 2 mg. of digitoxin added to 500 cc. of urine can be recovered.

Behavior of Digitoxin in the Organism

The heart-mass, according to Cloetta and Fischer (l.c.), possesses distinctly though in slight degree the power of digitoxin fixation. The quantity of poison adsorbed increases with the weight of tissue used and with the duration of action. Other organs, particularly the liver, manifest the same behavior. Experiments extending over a longer period disclosed the fact that the heart exhibits for digitoxin a distinct attractive power which asserts itself only very slowly. Because of the possibility that heart muscle might have the power of destroying the poison, Cloetta and Fischer studied the behavior of digitoxin as to decomposition by first attempting to destroy it by means of protoplasm and oxidative influences. These experiments showed that digitoxin is capable of resisting isolated tissue as well as the action of hydrogen peroxide, emulsin and invertin. In acute poisonings the poison could also be found again in the animal body. Part of the digitoxin was eliminated though slowly by way of the urine. Digitoxin even manifests a pronounced cumulative action!

Examination of parts of the cadaver for the various principles in digitalis is confined to detection of digitoxin in parts of organs and in urine, since the other digitalis glucosides apparently completely disappear in the organism. Holste[1] studied the action at 37° of different ferments, that is, of diastase, emulsin, pancreatin, trypsin, as well as saliva, gastric and intestinal juice, upon digitalis glucosides, strophanthin, and fresh digitalis infusion. He tested at various intervals of time their activity upon the frog's heart. Following a rather long action of these ferments, digitalis infusion was rendered completely inactive. After shorter action a decrease in activity was observed. Gastric juice and pancreatin had the strongest action. Digitoxin, crystallized digitalin and strophanthin exhibited higher powers of resistance. The uncertainty of digitalis therapy may therefore be attributed, in part at least, to cleavage of the active constituents of digitalis by digestive ferments.

Physiological Detection of Digitalis and Other Substances Having Digitalis Action

Digitalis displays a characteristic paralyzing action upon heart muscle, especially upon the frog's heart, and even in traces may be recognized by this action. For tests upon the entire animal, green frogs are to be preferred on account of their greater sensitiveness to substances having digitalis action. Test animals should be healthy, lively and weigh 30–35 grams. Experiments of this character should be made only by professional pharmacologists or by physicians who have had the necessary training and experience. Four nooses of thick cotton twine are attached to the legs of the experimental frog and then drawn through the slits of the frog-board as shown in Fig. 29.[2]

By means of curved, pointed scissors cut downward in the region of the arms a small flap of skin. Then grasp with forceps from the side the lower cartilaginous part of the sternum and cut upward a small rectangular opening. Now make a longitudinal incision in the exposed pericardium and press lightly upon the abdomen in proximity to the ventricle. As a result the heart ventricle is pressed out and lies free as shown in the sketch. The opening should be just large enough to expose the ventricle. If it is larger the liver may also be pressed out, especially in case of movement. By means of a hypodermic syringe inject about 1 cc. of an aqueous, alcohol-free solution of the substance in such a manner that about half the solution is quickly introduced into the upper part of one thigh and the remainder into the other thigh. This is done by inserting the fine needle of the syringe, made as sharp as possible, a little below the knee into the lymph-sac in the lower part of the thigh and carefully forcing it from there close under the skin to about the middle of the lymph-sac in the upper part of the thigh. Quickly withdraw the needle after injection and record the time. Then watch for the appearance of systolic stasis of the heart and record the time required. The heart will undergo considerable diminution in size. The duration of the experiment should not be more than 30 minutes. If systole fails to appear during this time, repeat

[1] A. Holste: Behavior of the Substances of the Digitalin Group toward Ferments (Enzymes). Archiv. f. exper. Pathol. u. Pharmakol. 68 (1912), 323.

[2] From H. Führer: "Detection and Estimation of Poisons Biologically," Urban and Schwarzenberg, 1911, page 71.

the experiment upon a new experimental frog, increasing somewhat the quantity of substance injected.

This biological test may be used for the recognition not only of digitoxin but of other "heart poisons," such as strophanthin, convallamarin, scilla, helleborein, oleandrin and adonidin. All these substances exhibit specific "digitalis action."

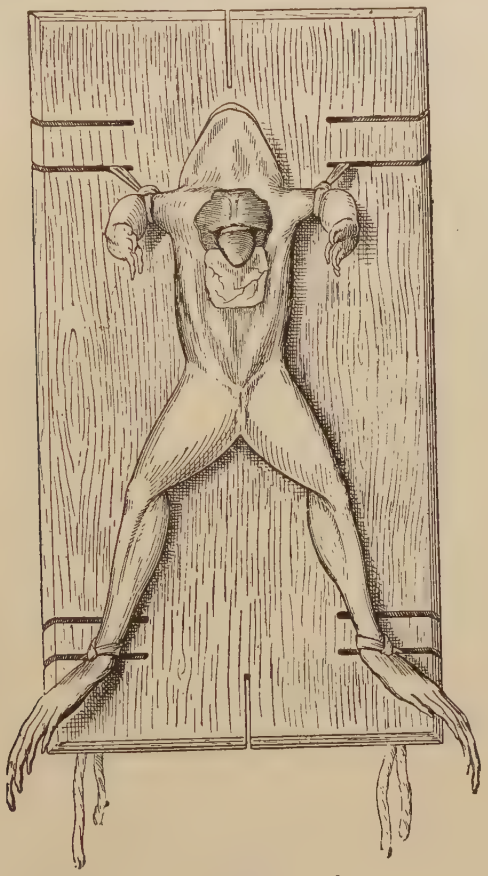


FIG. 29.—Frog board.

DIGITONIN

Digitonin, $C_{55}H_{90}O_{29}$, is a saponin obtained from the seeds of Digitalis purpurea. At most the leaves of the digitalis plant contain only traces. From alcohol it crystallizes in fine needles and in the pure condition is soluble in water with difficulty. When shaken its aqueous solutions foam strongly. It is insoluble in ether and soluble

in 50 parts of 50 per cent. alcohol. At 225° it sinters and softens at 235° with yellow color. Pure digitonin gives no color with concentrated hydrochloric acid, nor with the Kiliani-Keller reagent for digitoxin. But a red color is developed with concentrated sulphuric acid and increases in intensity upon addition of a trace of bromine water. Digitonin is not a heart-poison but as a saponin manifests toxic action.

Digitonin-cholesteride has the formula $C_{82}H_{136}O_{30} = (C_{55}H_{90}O_{29}.C_{27}H_{46}O)$. Windaus[1] has furnished proof that saponin substances through addition of cholesterol lose their property of dissolving blood-corpuscles. This cholesterol action moreover is ascribed to formation of well characterized saponin-cholesterides. Digitonin in particular easily gives a beautifully crystallizing cholesteride of the above composition when alcoholic solutions of cholesterol and digitonin are mixed. The precipitate that forms consists of fine needles. But larger crystals of digitonin-cholesteride may be obtained if a little water is added to its solution in boiling methyl alcohol; 100 cc. of methyl alcohol at 18° dissolve 0.47 gram of cholesteride. Digitonin-cholesteride is insoluble in cold water, acetone, ether, acetic ether and benzene. The air-dried product contains crystal water which it loses at 110°. In this condition it is very hygroscopic. It has no melting-point but gradually undergoes decomposition when heated above 240°. This cholesteride heated with methyl alcohol is decomposed into its constituents. If it is boiled for 4 hours with this alcohol, then extracted with water and ether, and the ether layer washed with water, pure cholesterol remains when the ether is evaporated. The cholesteride may also be decomposed by hot xylene which takes up cholesterol but not digitonin. Decomposition of 10 grams of digitonin-cholesteride is complete after 10 hours extraction with hot xylene in Stork's apparatus. Digitonin-cholesteride may be used for the quantitative determination of cholesterol and phytosterol. Since cholesterol esters occurring in the animal organism are not precipitated by digitonin, the digitonin method may be used to differentiate and determine quantitatively free cholesterol in presence of cholesterol esters in blood, blood-serum, bile and organs. Phytosterols, plant sterols that also act as antidotes for saponins, give similar complex compounds with digitonin. Digitonin and phytosterol in alcoholic solution form sitosterol-digitoninphytocholesteride, $C_{82}H_{136}O_{30} = (C_{55}H_{90}O_{29}.C_{27}H_{46}O)$. It crystallizes in fine needles and closely resembles digitonin-cholesteride in properties.

Chemistry of Digitonin.—Notwithstanding the ease with which it crystallizes, digitonin can be completely separated only with great difficulty from the other glucosides also present in the seed. This fact explains why crystallized digitonin was not found, until many years after its discovery, to be mixed with about 15 per cent. of gitonin. In the light of this fact, it is easy to understand how the work of Kiliani and his students was carried out with a mixture of different

[1] A. Windaus: Cholesterol as an Antidote for Saponins. Ber. d. Deutsch. chem. Ges. 42 (1909), 238. Quantitative Estimation of Cholesterol and Cholesterol Esters in Some Normal and Pathological Kidneys. Zeitschr. f. physiol. Chem. 65 (1910), 110.

digitalis saponins and how the transformation products described as coming from digitonin were in reality derived only in part from that glucoside and in part from the other glucosides present (Windaus and Weil[1]). These two investigators have explained the chemistry of digitonin after careful examination of esters that do not so easily give addition-products, especially by determining methoxyl and by their saponification, and also by employing new methods, that is, by determining according to Zerewitinoff[2] the number of reactive hydrogen atoms contained in a molecule. According to Windaus and Weil, hydrolytic cleavage of digitonin by alcoholic hydrochloric acid into digitogenin, galactose, glucose and a pentose probably takes place as shown in the following equation:

$$\underset{\text{Digitonin}}{C_{55}H_{90}O_{29}} + 5H_2O = \underset{\text{Digitogenin}}{C_{26}H_{42}O_5} + 4\underset{\substack{\text{Galactose-}\\\text{glucose}}}{C_6H_{12}O_6} + \underset{\text{Pentose}}{C_5H_{10}O_5}$$

Pure, crystallized digitogenin heated with acetic anhydride gives a triacetyl-derivative. Upon the basis of their experimental results derived from the latter product, which is also crystalline, Windaus and Weil have assigned to digitogenin the formula $C_{26}H_{42}O_5$ and in part determined its constitution. It is a neutral substance containing 3 hydroxyl-groups. It contains neither a methoxyl nor a lactone group, nor can aldehyde or ketone groups be detected. The two oxygen atoms not present as hydroxyl-groups are probably in an oxide-like union. The fact that this substance of high molecular weight can be distilled *in vacuo* without decomposition deserves mention. Oxidation of digitogenin by chromic acid gives crystalline digitogenic acid which dried *in vacuo* quite likely has the formula $C_{26}H_{38}O_7.\frac{1}{2}H_2O$. On account of undergoing change at 100°, it cannot be dried and with diazo-methane solution gives a crystalline dimethylester, $C_{26}H_{36}O_7(CH_3)_2$. By saponifying this and other esters of digitogenic acid with alcoholic potassium hydroxide, digitoic acid, $C_{26}H_{38}O_7$, a stereo-isomer of digitogenic acid is obtained. Free digitogenic acid is also a very labile substance and gives, when warmed to 100° or when boiled with dilute caustic alkalies, β-digitogenic acid and digitoic acid. As stated above, crude digitonin contains gitonin. Digitogenin prepared from it contains gitogenin as an impurity and upon oxidation gives a mixture of digitogenic acid and gitogenic acid. According to Windaus and Weil, the three acids, gitonic, desoxy-digitogenic and hydro-digitoic acid, obtained by Kiliani from such an impure digitogenic acid, are identical with gitogenic acid.

Careful oxidation of digitogenic acid with potassium permanganate gives tribasic oxy-digitogenic acid, $C_{26}H_{38}O_9$. This acid is also obtained in the same manner and with the same yield from digitoic acid and β-digitoic acid, since 2 atoms of oxygen are required for 1 molecule of acid:

$$\underset{\substack{\text{Digitogenic}\\\text{acid}}}{C_{26}H_{38}O_7} + O_2 \rightarrow \underset{\substack{\text{Oxy-digitogenic}\\\text{acid}}}{C_{26}H_{38}O_9}$$

This is proof of the fact that digitogenic acid, digitoic acid and β-digitogenic acid are isomeric.

[1] A. Windaus and K. Weil: Digitonin and its Cleavage-products. Zeitschr. f. physiol. Chemie, Bd. 21 (1922), 62.

[2] T. Zerewitinoff: Quantitative Estimation of Hydroxyl Groups by Magnesium Organic Compounds. Ber. d. Deutsch. chem. Ges. 40 (1907), 2023.

The methyl ester, $C_{25}H_{36}O_6(OCH_3)$, of oxy-digitogenic acid is formed by shaking this compound with diazo-methane. The methyl esters of the oxy-digitogenic acids obtained from the three acids, digitogenic, digitoic and β-digitogenic acid, were found to be identical.

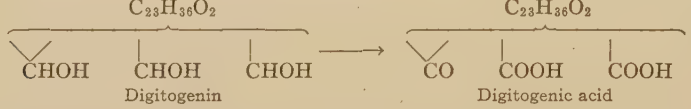
Digitic acid, $C_{26}H_{38}O_{10}$, obtained by oxidizing digitogenic acid, digitoic acid, or oxy-digitogenic acid with strong alkaline potassium permanganate solution (Kiliani) differs from the latter acid in containing one more atom of oxygen present as hydroxyl:

$$\underset{\text{Oxy-digitogenic acid}}{C_{26}H_{38}O_9} + O \rightarrow \underset{\text{Digitic acid}}{C_{26}H_{38}O_{10}} = C_{26}H_{37}O_9(OH)$$

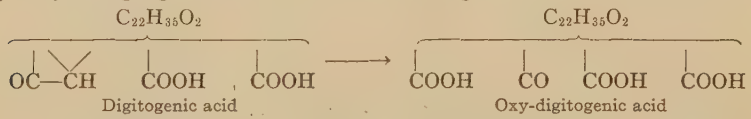
With glacial acetic acid and hydrochloric acid even at 0°, digitic acid loses a molecule each of carbon dioxide and water and passes into "anhydro-digitic acid," $C_{25}H_{36}O_7(?)$. Windaus and Weil draw the following conclusions from the results of their experiments as to the constitution of digitonin and its derivatives.

Digitogenin, $C_{26}H_{42}O_5$, is a saturated triatomic alcohol the two remaining oxygen atoms of which are present in an oxide-like union. Upon the basis of the number of hydrogen atoms present, digitogenin like cholesterol contains four hydrogenized rings.

Digitogenic acid, $C_{26}H_{38}O_7$, obtained by the oxidation of digitogenin is a dibasic ketone acid. In all probability a secondary alcohol group is oxidized to the ketone group and at the same time two secondary alcohol groups in the α-position in one ring are converted into two carboxyl groups with breaking of the ring:

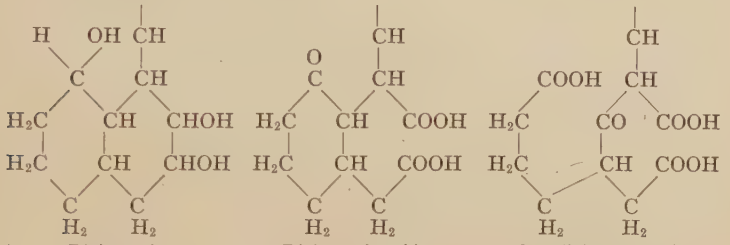


In the conversion of digitogenic acid into tribasic oxy-digitogenic acid, $C_{26}H_{38}O_9$, the group (CO—CH) held in ring-form is apparently oxidized to carboxyl and carbonyl with the opening of a second ring. Consequently the third hydroxyl of digitogenin is also attached to the ring:



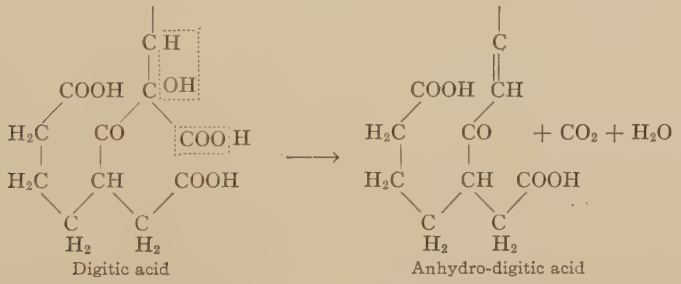
Since the isomeric acids, digitogenic acid and digitoic acid, give the same oxy-digitogenic acid, the supposition is probable that they differ only in the spatial arrangement of the hydrogen atoms in the radical $-\overset{|}{\underset{|}{C}}H$, corresponding to the conversion of digitogenic acid into digitoic acid:

Oxy-digitogenic acid differs from digitoic acid in losing carbon dioxide rather easily, a characteristic both of β-ketone and malonic acids. In this way it was shown that the newly formed ketone or carboxyl group of oxy-digitogenic acid stands in the β-position to one of the carboxyls present, and further that the three hydroxyls of digitogenin are attached to two rings joined together as represented in the following formulae:



Digitogenin — Digitogenic acid — Oxy-digitogenic acid

Digitic acid, $C_{26}H_{38}O_{11}$, an oxy-keto-tricarboxylic acid containing a tertiary hydroxyl, is formed by further oxidation of oxy-digitogenic acid. It loses a molecule of water very easily and then even at 0° a molecule of carbon dioxide, passing into anhydro-digitic acid, a mono-keto-dicarboxylic acid:



Digitic acid — Anhydro-digitic acid

STROPHANTHIN

The seeds of various species of Strophanthus indigenous to West Africa contain a substance having a bitter taste, a strophanthin. These substances collectively exhibit a typical action upon the heart but they differ from one another in the intensity of this action. If the practice of Thoms[1] is adopted, the various strophanthins are distinguished by placing before the word strophanthin the small initial letter of the name of the particular Strophanthus from which it comes. In accordance with this plan the strophanthin from Strophanthus gratus is designated g-strophanthin, that from Strophanthus hispidus, Kombé and emini, h-, k- and e-strophanthin. Chemically and physiologically these strophanthins of different origin are not identical.

To isolate the "strophanthins," extract with alcohol finely ground Strophanthus seeds that have first been extracted with petroleum ether or carbon

[1] H. Thoms: The Strophanthin Question from the Chemical Standpoint. Ber. d. Deutsch. pharmaz. Ges. 14 (1904), 104.

disulphide to remove fat. Distil the alcohol from the extract, dissolve the residue in water, and precipitate the filtered solution with excess of tannic acid. Collect the precipitate, which contains strophanthin, and wash with water. Then while still moist mix with excess of basic lead acetate, dry this mixture at moderate heat, and extract with alcohol. Free the filtered extract from lead with hydrogen sulphide and expel the latter from the filtered solution. Remove color with animal charcoal and evaporate the solution; or precipitate strophanthin from its alcoholic solution by adding ether in large excess.

Chemistry of Strophanthins.—According to Feist,[1] anhydrous Kombé-strophanthin has the formula $C_{40}H_{66}O_{19}$. Hydrolyzed by means of 0.5 per cent. hydrochloric acid, it breaks down at 70–75° into strophanthidin, which separates as a crystalline meal, and a complicated carbohydrate called strophanthobiose-methyl ether:

$$C_{40}H_{66}O_{19} = \underset{\text{Strophanthidin}}{(C_{27}H_{38}O_7.2H_2O)} + \underset{\text{Strophanthobiose-methyl ether}}{C_{13}H_{24}O_{10}}$$

The latter compound, boiled under a reflux with 1 per cent. sulphuric acid, undergoes hydrolytic cleavage into methyl alcohol, rhamnose and mannose:

$$C_{13}H_{24}O_{10} + 2H_2O = CH_3.OH + \underset{\text{Mannose}}{C_6H_{12}O_6} + \underset{\text{Rhamnose}}{C_6H_{12}O_5}$$

Strophanthin-Kombé is a fine crystalline meal containing a variable quantity of crystal water, usually $C_{40}H_{66}O_{19}.3H_2O$. In the anhydrous condition it melts at 170°. It is easily soluble in water, less soluble in alcohol, almost insoluble in chloroform, and insoluble in ether. In 5.2 per cent. aqueous solution $[\alpha]_D = +10.12°$.

Detection of k-Strophanthin

1. Sulphuric Acid Test.—Concentrated sulphuric acid at once colors k-strophanthin deep emerald-green. Upon standing in air this test becomes discolored. Gadamer recommends 80 per cent. sulphuric acid for this test.

2. Reduction Test.—Unlike many other glucosides, strophanthin does not reduce Fehling's solution even when heated but does reduce silver nitrate.

3. Ferric Chloride Test.—If a trace of ferric chloride is added to an aqueous solution of k-strophanthin and then concentrated sulphuric acid, a red-brown precipitate is formed. Immediately, or after 1–2 hours, it becomes emerald-green.

g-Strophanthin, $C_{30}H_{40}O_{12}.9H_2O$.—Thoms (l.c.) obtained about 3.6 per cent. of this strophanthin from the seeds of Strophanthus gratus indigenous to the Cameroon. The powdered seeds were first treated with petroleum ether to remove fat and then exhausted with 96 per cent. alcohol. Alcohol was removed from the percolate and the crystals that separated after long standing were recrystallized from hot water. g-Strophanthin forms shining plates melting at 187–188° when anhydrous. It is very easily soluble in hot water, soluble at 15° in 30 parts of absolute alcohol, in 30,000 parts of chloroform, and in 52,000 parts of ether. Concentrated sulphuric acid colors g-strophanthin red. This

[1] F. Feist: Strophanthin and Strophanthidin. Ber. d. Deutsch. chem. Ges. 31 (1898), 534; 33 (1900), 2063, 2069, 2091.

color upon addition of water changes to green with separation of greenish white flocks Heated with dilute hydrochloric or sulphuric acid, it undergoes hydrolytic cleavage into g-strophanthidin and rhamnose.

Detection of g-Strophanthin

If a solution of g-strophanthin, about 0.01 gram in 1 cc. of water, is carefully added as an upper layer to concentrated sulphuric acid, the latter becomes rose to red and the water layer dirty green. Practically nothing is known with regard to the presence of strophanthin in the animal body and its elimination; neither is it known whether this substance can still be detected in the cadaver following poisoning by strophanthin. As in the case of the digitalis glucosides, the physiological test is the best method of recognizing strophanthin.

Strophanthidin.—Air-dried strophanthidin probably has the formula $C_{27}H_{38}O_7 \cdot 2\frac{1}{2}H_2O$. Over ignited sand $\frac{1}{2}$ molecule of water can be removed. One and two molecules of water can be successively expelled in desiccator over concentrated sulphuric acid, or at 110–125°. It persistently retains crystal water even after recrystallization from methyl or ethyl alcohol. Like strophanthin, crystals of strophanthidin dried as above eagerly absorb water from air without deliquescing. It melts at 169–170°, foams at 176°, solidifies on cooling, and then does not melt until 232°. Strophanthidin is easily soluble in alcohol, acetone, and glacial acetic acid; difficultly soluble in ether, chloroform and benzene; and insoluble in ligroin. It crystallizes from methyl alcohol in beautiful, shining prisms that are frequently united in the form of radiating crystalline warts. $[\alpha]_D = +45.45°$. Strophanthidin is a neutral substance insoluble in water and sodium carbonate solution. On the contrary, it is soluble warm in caustic alkalies and in barium hydroxide solution. In this respect it behaves like a di-lactone. Strophanthidin dissolves in concentrated sulphuric acid with brick-red color.

Colorimetric Estimation of Strophanthin

(Autenrieth and Quantmeyer[1])

Strophanthin belongs to the class of saponins that act like digitalis (see page 437). Inasmuch as no very satisfactory chemical method of estimating strophanthin is known, pharmacologists use the biological method almost exclusively. The usual custom is to observe the time that elapses from the moment the given solution is injected into the two lymph-sacs in the upper thighs of a frog until the heart is brought to systolic stasis. In this method, as worked out chiefly by Focke for the estimation of "heart poisons," time is the principal factor, whereas Gottlieb determines the smallest quantity that will produce systolic heart-stasis in a large number of experimental frogs within 30 to 45 minutes at most. The best of the chemical methods of estimating strophanthin should be that of Fromme who subjects this substance to hydrolytic cleavage by acid and weighs strophanthidin obtained. The objection to this method is the impossibility of making an accurate quantitative separation of strophanthidin from the large amount of fat present in Strophanthus seeds. The color reaction, however, given by officinal Kombé-strophanthidin with concentrated sulphuric acid is well suited for colorimetric determination. Acetic anhydride is a suitable anhydrous solvent to use for strophanthin. Over a concentration range of 0.07–0.40 mg.

[1] Not as yet published by the authors.

of strophanthin per cc. of solution the tinctorial strength is exactly proportional to the quantity of strophanthin.

Requirements.—1. Purest acetic anhydride having a boiling-point of 136.4°. This reagent will remain unchanged for a long time, if kept tightly closed in dark glass-stoppered bottles. 2. Purest concentrated sulphuric acid. 3. A 5 or 10 cc. glass-stoppered graduated cylinder. 4. Purest Kombé-strophanthin (Merck) for calibration of the comparison-wedge.

Calibration of Comparison-Wedge and Construction of Calibration Curve

Fill the comparison-wedge with a permanent, artificial solution prepared from chrome alum. This solution has a green color identical with that of a solution of strophanthin in sulphuric acid. For calibrating the wedge use a colorless solution of 1 mg. of strophanthin (4) in 1 cc. of acetic anhydride. With a capillary pipette accurately measure different amounts of strophanthin solution into the small glass-stoppered graduate (3), cool and drop in 1 cc. of concentrated sulphuric acid, bring the volume to 5 cc. with acetic anhydride and shake. After 5–10 minutes the intensity of color has reached a maximum. Then introduce a portion of this solution into the glass trough of the colorimeter, and determine the point where the color strength is the same as that of the comparison-wedge, and each time note

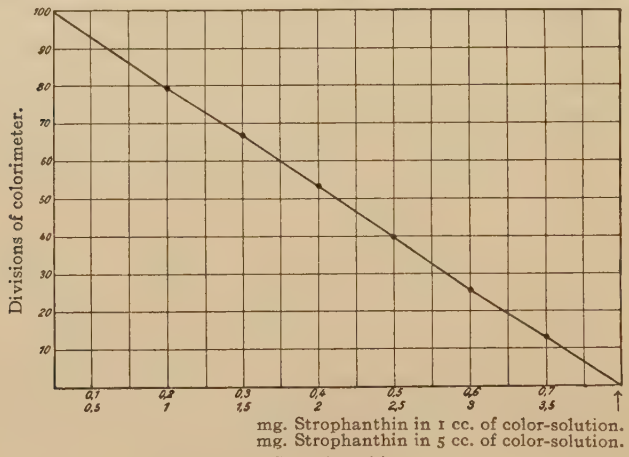


FIG. 30.—Strophanthin curve.

the reading on the scale. The mean of these readings is taken as the final result. The shade and strength of the green solutions remain unchanged for about 30 minutes and then gradually pass into olive-green, dirty brown and gold-brown. The following results were obtained in the calibration of a comparison-wedge:

Strophanthin solution in cc.....	1	1.5	2	2.5	3	3.5
Strophanthin (mg.) per 5 cc. of color solution...	1	1.5	2	2.5	3	3.5
Strophanthin (mg.) per 1 cc. of color solution.	0.2	0.3	0.4	0.5	0.6	0.7
Scale reading for equal color strength.....	80	67	53	39	25	13

If the quantities of strophanthin in mg. are recorded upon the abscissae of a coördinate system and the readings for equal color strength upon the ordinates, the above curve for the comparison-wedge may be constructed.

This comparison-wedge therefore has a measuring range from 0.15–0.75 mg. of strophanthin per cc. of color solution. In case the color solutions appear too dark, dilute them with acetic anhydride and in the calculation take into consideration the degree of dilution.

Estimation of Strophanthin in Tincture of Strophanthus

In a small porcelain evaporating dish evaporate 2 cc. of tincture to dryness upon the water-bath. Stir the residue in the dish while still warm with 5 cc. of acetic anhydride and pour the solution through a dry filter. Strephanthin easily passes into solution but other coloring matters in the tincture remain undissolved. Introduce 1, 1.5 or 2 cc. of this acetic anhydride solution into a small glass-stoppered graduate (3), dilute with acetic anhydride to 4 cc., cool and drop in 1 cc. of pure concentrated sulphuric acid. After 5–15 minutes, when the intensity of color has reached a maximum, introduce some of the solution into the small glass trough and note the scale-reading at the point where its color strength is the same as that of the calibrated comparison-wedge. Finally ascertain the corresponding strophanthin value on the calibration curve of the comparison-wedge.

Example.—Tincture taken = 2 cc. Solution of the residue from evaporation in acetic anhydride = 5 cc. Used for the colorimetric determination 1 cc. = 0.4 cc. of tincture. Scale reading for equal color strength is 67 = 0.29 mg. in 1 cc., or 1.45 mg. of strophanthin in 5 cc. of color solution or in 0.4 cc. of tincture. So 100 grams of tincture of strophanthus contain $250 \times 1.45 = 362.5$ mg. or 0.3625 gram of strophanthin. The quantity of strophanthin found in samples of tincture of strophanthus taken from different apothecaries in Freiburg ranged from 0.32 to 0.39 per cent.

To estimate strophanthin in Strophanthus seed, according to directions given in the German Pharmacopoeia, IV Edition, officinal tincture of strophanthus is prepared by extracting the seeds powdered mediumly fine with 10 parts of diluted alcohol (sp. gr. 0.892–0.896 = 69–68 volumes, or 61–60 parts by weight of alcohol in 100 parts) and strophanthin in this tincture is then determined colorimetrically by the method described above. Preliminary removal of fat from the seeds is not permitted since some strophanthin may be lost in this way. The mean of several determinations of strophanthin in Strophanthus seed by this method was 3.15 per cent., whereas the same seed, after removal of fat with ether or petroleum ether, or by direct extraction with water, gave a yield of only 2.16 per cent.

TOAD POISON

By extracting skins of toads with alcohol, Wieland and Weil[1] obtained in conjunction with suberic acid, cholesterol and fatty acids (solid and liquid) a poisonous substance, bufotalin ($C_{26}H_{36}O_6$), appearing in colorless crystals and having an action like that of digitalis. The yield of crude bufotalin before crystallization averaged 20 grams from 2000 toads. According to the latest results obtained by Wieland and Alles,[2] the nitrogenous substance called bufotoxin, hav-

[1] H. Wieland and F. J. Weil: Poison from Toads. Ber. d. Deutsch. chem. Ges. 46 (1913), 3315.

[2] H. Wieland and R. Alles: The Toxic Substance from Toads. Ber. d. Deutsch. chem. Ges. 55 (1922), 1789.

ing the formula $C_{40}H_{62}N_4O_{11}$, is the primary toxic substance from our native toads and was isolated from the fresh secretion of the cutaneous glands. This secretion may be obtained by squeezing the cutaneous glands, that is, the so-called parotis of living toads with forceps having a dull edge. The nearly white milky juice procured in this way from 380 toads, when dried in a vacuum desiccator, gave 4 grams of a hard vitreous mass. Bufotoxin recrystallized several times from alcohol forms colorless druses made up of fine needles melting at 204–205°. Fusion of this compound is attended with vigorous decomposition. It is practically insoluble in water, ether, acetone, chloroform, acetic ether, and petroleum ether; difficultly soluble in absolute alcohol; more easily soluble in 50 per cent. alcohol; and very readily soluble in methyl alcohol and pyridine. Pure bufotoxin is not decomposed after 80 hours boiling in alcoholic solution. It is insoluble in dilute mineral acids, thus exhibiting no basic properties.

Color Reactions (Cholesterol Test).—Dissolve bufotoxin in about 2 cc. of chloroform. Add first a few drops of acetic anhydride and then concentrated sulphuric acid drop by drop. Temporarily the mixture has a red color which changes to blue and finally becomes a beautiful green. Minute quantities of toad poison may be detected by this test. Bufotoxin and bufotalin both give this test but in the case of bufotoxin the first cherry-red phase is even more fugitive than with bufotalin. Bufotalin dissolves in concentrated sulphuric acid with an orange-red color that changes on standing to deep red and exhibits distinct green fluorescence. Cholic acid behaves very much in the same way.

Constitution.—Bufotalin, obtained by Wieland in his earlier experiments with extracts from toad skins, might have come into existence as a kind of "genin" from the bufotoxin primarily present, since the latter is exceedingly sensitive to acids. When boiled for 4–5 hours with dilute alcoholic hydrochloric acid, it readily undergoes hydrolytic cleavage into bufotalien, $C_{24}H_{30}O_3$, and suberyl-arginine, $C_{14}H_{26}N_4O_5$. The bufotalien that separates crystallizes from alcohol in light yellow leaflets melting at 219°. Suberyl-arginine, isolated as such with difficulty, can be decomposed when boiled with strong hydrochloric acid into suberic acid, melting at 139–140°, and arginine. The latter was recognized by its nitrate and picrolonate (mpt. 230°). According to Wieland and Alles, conversion of bufotoxin into bufotalin and the hydrolytic cleavage of the former may be expressed by the following equations:

I. $C_{26}H_{37}O_6 - O.CO.C_{13}H_{25}N_4O_3 \rightarrow C_{26}H_{36}O_6 + C_{14}H_{26}N_4O_5$

Bufotoxin — Bufotalin — Suberyl-arginine

II. $C_{40}H_{62}N_4O_{11} \rightarrow C_{24}H_{30}O_3 + C_{14}H_{26}N_4O_5 + H.C_2H_3O_2 + H_2O$

Bufotoxin — Bufotalien — Suberyl-arginine — Acetic acid

III. $HN{:}C(NH.CO.(CH_2)_6.COOH)(NH.(CH_2)_3.CH(NH_2).COOH) + H_2O = HOOC.(CH_2)_6.COOH +$

Suberyl-arginine — Suberic acid

$HN{:}C(NH_2)(NH.(CH_2)_3.CH(NH_2).COOH)$

Arginine

According to Wieland, bufotalin, $C_{26}H_{36}O_6$, the nucleus of bufotoxin, is a derivative of a molecule containing 24 carbon atoms which like the bile-acids may be composed of four hydro-aromatic rings. The remaining two carbon atoms in bufotalin make up an acetyl-group present in the form of an ester. This group may be removed by hydrolysis as acetic acid. Bufotalin is also a lactone and contains two hydroxyl-groups, one secondary and the other tertiary. Since bufotalin adds four atoms of hydrogen when subjected to catalytic hydrogenization and is converted into the saturated hydro-bufotalin, $C_{26}H_{40}O_6$, two ethylene linkings must be present in the molecule. When treated with concentrated hydrochloric acid, bufotalin loses a molecule each of acetic acid and water, becoming bufotalien, $C_{24}H_{30}O_3$, which has a yellow color and contains four ethylene linkings:

$$\underset{\text{Bufotalin}}{C_{26}H_{36}O_6} = \underset{\text{Bufotalien}}{C_{24}H_{30}O_3} + \underset{\text{Acetic acid}}{H.C_2H_3O_2} + H_2O \qquad \text{IV.}$$

Bufotalien is the bridge to the bile-acids, since hydrogenization (+ 8 H-atoms) converts it into the saturated bufotalan, $C_{24}H_{38}O_3$, which still contains the lactone-group. The oxy-acid, $C_{24}H_{40}O_4$, corresponding to this lactone, is isomeric with desoxy-cholic acid obtained from the bile-acids:

$$\underset{\text{Bufotalien}}{C_{24}H_{30}O_3} \xrightarrow{+8H} \underset{\text{Bufotalan}}{C_{24}H_{38}O_3} \xrightarrow{+H_2O} \underset{\text{Oxy-acid}}{C_{24}H_{40}O_4}$$

Desoxy-cholic acid may be regarded as a dioxy-cholanic acid. Cholanic acid, $C_{24}H_{40}O_2$, is probably present as a common basal substance in bufotalin, the first cleavage-product of bufotoxin from toad poison, as well as in the bile-acids and cholesterol. Like cholesterol the bufotoxin molecule contains one ethylene linking.

Physiological Action.—Both bufotoxin and bufotalin exhibit pronounced digitalis action, bringing the frog's heart to systolic stasis and also having strong haemolytic action. In this respect the two toad poisons behave like saponins.

SAPONINS OR SAPONIN SUBSTANCES

The term saponins, or saponin substances, includes a large number of glucoside-like substances of widespread occurrence in the plant kingdom having in common certain chemical, physical, and especially physiological properties. When shaken, their aqueous solutions readily foam. In this respect they resemble soaps. Many saponin substances have a sharp, harsh taste. In powdered form they excite violent sneezing. They are capable of holding many finely divided substances in a state of emulsion. Saponin substances dialyze slowly and very incompletely. They may be partially salted out from their solutions. Excepting the gluco-alkaloid solanine, which contains nitrogen and is alkaline, saponins may be classified chemically as nitrogen-free glucosides.

At the present time, chemical knowledge of saponins and of their hydrolytic cleavage-products is still very defective, because of the extreme difficulty involved in getting them pure and establishing their exact identity on account of their amorphous character.

With few exceptions saponins dissolve easily in water and their solutions foam strongly. They are also colloidal and possess an emulsifying action. Saponins dissolve with tolerable ease in hot dilute alcohol and in methyl alcohol; only very slightly in absolute alcohol and acetone; and not at all in ether and petroleum ether. Saponins dissolve with relative ease in phenol and less easily in isobutyl and isoamyl alcohol. Some saponin solutions are optically active others inactive. The application of drugs containing saponins in washing, in preventing finely divided substances from settling, and crystalline substances from crystallizing depends upon the emulsifying properties of saponin-substances. Some saponins are feebly acid in character. These are the saponin-acids. Other saponins react neutral and are chemically indifferent. These are the sapotoxins. Frequently saponin-acids and sapo-toxins occur together in one and the same plant. Consequently they must be related to one another. Reagents used to precipitate saponins from concentrated aqueous solution are barium hydroxide in saturated aqueous solution; neutral and basic acetate of lead; and ammonium sulphate.

Like soaps and many proteins, saponins may be salted from aqueous solution by ammonium sulphate and various other soluble salts. Saponins to a great extent are separated from solution by animal charcoal and by lead sulphide as a result of adsorption. Boiling alcohol will redissolve what has been adsorbed. Saponins also exert a solvent action upon red blood-corpuscles. They destroy the stroma of the corpuscles and haemolysis takes place. Saponins withdraw lecithin, and partially also cholesterol, forming with them labile molecular compounds and bringing the blood-corpuscles into solution. (See "Physiological Action of Saponins.")

Hydrolytic cleavage of saponins by dilute acids (3–4 per cent. sulphuric acid), which frequently runs to completion very slowly, usually gives amorphous compounds called sapogenins. At the same time certain sugars, such as glucose, galactose and pentoses, are formed. Some sapogenins are crystalline but in general they are even more difficult to purify than saponins, for their composition seems to depend upon external experimental conditions.

Occurrence of Saponins.—Evidence of the wide distribution of saponins in the plant kingdom is afforded by the fact that up to the present time more than 200 saponin-substances have been found in over 50 plant families including mono- and dicotyledonous plant species. The parts of plants containing saponins are roots (Senega, Saponaria), tubers (Cyclamen), barks (Quillaja, Guaiacum), fruits (Sapindus, Saponaria), seeds (Aesculus, Agrostemma, Thea), stems (Dulcamara), and leaves (Guaiacum). In fact there is scarcely any part of the plant organism in which saponins may not occur. The plant families producing saponin substances more abundantly are the caryophyllaceae, colchicaceae, polygalaceae, sapindaceae and sileneae. Quite considerable quantities of saponins may occur in the particular part of the plant. In the following some of the exhaustively investigated saponins, differentiated according to origin, will be mentioned:

Saponin from Soapwort.—Saponaria officinalis contains 4–5 per cent. of saponin. E. Schmidt isolates it by extracting finely ground soapwort with 90–91 per cent. alcohol, collecting the precipitate that separates from the hot filtered extracts after 24 hours, washing with ether and drying. This precipitate is

purified by dissolving in a little water and reprecipitating with barium hydroxide. Precipitated barium hydroxide saponin is washed with barium hydroxide solution and decomposed with carbon dioxide. The filtered aqueous solution is evaporated and saponin precipitated by alcohol and ether. This saponin is a white, amorphous powder dissolving readily in water, slightly in alcohol and insoluble in ether.

Quillaja-Saponins.—The bark of Quillaja saponaria contains as high as 8.8 per cent. of saponins. Quillajac acid, which Kobert designates as the physiologically active toxic modification of Quillaja-saponins, is characterized by a strong and persistently harsh taste. Neutral lead acetate precipitates it from aqueous extracts of Quillaja bark. Basic lead acetate added to the filtrate from this precipitate causes further precipitation of a very poisonous compound, Quillaja sapotoxin! One and the same sapogenin has been obtained in crystalline form from quillajac acid and Quillaja-sapotoxin.

Saponins of Senega Root.—Polygalic acid and senegin, showing great similarity in behavior to quillajac acid and the sapotoxin, have been isolated from the root of Polygala senega.

Guaiac-Saponins.—The wood and bark of Guaiacum officinale contain two saponins, namely, guaiac-saponic acid precipitated by lead acetate and guaiac-saponin precipitated only by basic lead acetate. Both saponins are characterized by slight haemolytic properties and consequently are not very toxic.

Sapindus-Saponin.—This substance was isolated from the fruit of various species of Sapindus, for example, Sapindus Rarak DC (May[1]). The shells on the average contain 13.5 per cent. of saponin. It is a white, amorphous powder and very readily soluble in water. Barium hydroxide and basic lead acetate precipitate it but not quantitatively. It has a powerful haemolytic action. This saponin is hydrolyzed when warmed with 5 per cent. hydrochloric or sulphuric acid into a sapogenin, $C_{12}H_{18}O_3$, and one molecule each of a hexose and a pentose.

Winterstein and Maxim have shown that a naphthalene ring is the basis of the sapogenin from Sapindus as well as of that from the horse-chestnut, for these substances upon oxidation with nitric acid yielded a dinitro-naphthalene together with oxalic acid.

Saponin of Corncockle, Agrostemma-Sapotoxin.—Remove oil from coarsely powdered seeds of Agrostemma githago by means of ether and then extract for 2–3 days with 70 per cent. alcohol (5 liters for 2500 grams of seed). Evaporate the alcoholic extract upon the water-bath to the consistency of thick syrup and then stir with absolute alcohol. At first the residue passes into solution but further addition of alcohol precipitates the sapotoxin which soon becomes solid and pulverulent. Corncockle seed contains about 6.44 per cent. of fat. The yield of crude sapotoxin is 4.9–6.1 per cent., or at most 7.7 per cent. (Brandl[2]). Pure sapotoxin was obtained by a method of purification with lead acetate and basic lead acetate and extraction with alcohol. It is a light yellow, water-soluble

[1] O. May: Chemico-Pharmacological Investigation of the Fruit of Sapindus Rarak D. C. Archiv d. Pharmaz. 244 (1906), 251.

[2] J. Brandl: Sapotoxin and Sapogenin from Agrostemma Githago. Archiv. f. experim. Pathol. u. Pharmakol. 54 (1906), 245 and 59 (1908), 245.

powder giving no turbidity with lead acetate, a greenish white precipitate with basic lead acetate, and a yellowish precipitate with barium hydroxide solution. Concentrated sulphuric acid dissolves this sapotoxin with yellow color gradually changing to purplish red and exhibiting an absorption-band in the spectrum at E. The boiling solution reduces ammoniacal silver nitrate solution but has no action upon Fehling's solution! Three hours boiling with 4 per cent. sulphuric acid hydrolyzes it into a crystalline sapogenin and various sugars. Diluted 1:50,000, Agrostemma-sapotoxin causes haemolysis in a short time. Treated with cholesterol, Agrostemma-sapotoxin even to the extent of 0.01 gram fails to produce haemolysis. Injection of 12–15 mg. of this sapotoxin into the lymph-sac in the back of a frog paralyzes the animal within 3 hours.

Using Kobert's method of purifying saponins, Brandl isolated a new substance called agrostemmic acid from the precipitate caused by neutral lead acetate. This product constituted 6–7 per cent. of the crude sapotoxin. Agrostemmic acid is a brownish white powder differing from Agrostemma-sapotoxin principally in its behavior with lead acetate with which it gives a precipitate. This acid produces the same poisonous effects caused by the sapotoxin.

General Reactions and Detection of Saponins

1. **Foam Test.**—Aqueous saponin solutions when shaken foam more or less strongly as soap solutions do.

2. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves saponins with yellow color gradually changing to red, purplish red, and often red-violet or blue-green.

3. **Froehde's Test.**—With various saponins Froehde's reagent produces a blue-violet and frequently also a green color.

4. **Brunner-Pettenkofer Bile Acid Test.**—Since saponins are glucosides they give this test. According to Gadamer, the substance to be tested should be dissolved in a little water with a particle of purified ox-gall (Fel Tauri depuratum). This solution should then be added as an upper layer to an equal volume of concentrated sulphuric acid in a small test-tube. If a saponin is present, a blood-red zone will appear at the contact-surface of the two liquids and the entire mixture will become red when shaken.

Since nearly all sugars give the Brunner-Pettenkofer test, a positive result indicates a saponin only in absence of sugar. Glucosides, and consequently saponins, do not reduce Fehling's solution in the cold. Many sugars, however, that come into question do, and so it is frequently possible to decide by means of Fehling's test whether sugar or glucoside is present. In consequence of the cleavage of sugar as a result of hydrolysis by dilute acids, saponins have a reducing action upon Fehling's solution. In this respect cane-sugar behaves like a saponin. To detect saponin positively in presence of cane-sugar, the former should be extracted with an appropriate solvent or first precipitated with neutral or basic lead acetate and then set free from the precipitate with hydrogen sulphide.

5. **Haemolytic Test.**—If a solution of a saponin in 0.9 per cent. sodium chloride solution is added to 1 cc. of a mixture of 1 cc. of defibrinated blood and 100 cc. of 0.9 per cent. sodium chloride solution, red blood-corpuscles gradually dissolve and for this reason the originally turbid mixture becomes clear and laked.

6. **Fish Test.**—Most saponins exert a strong, specifically toxic action upon fish. Dilute with ordinary fresh water the solution to be tested for saponin and place in it a small fish. After 12–24 hours, if a saponin is present, the fish will become torpid and finally die.

Physiological Action of Saponins

Most saponin substances possess toxic properties. If introduced directly into the blood, their poisonous action is far greater than if taken internally by the mouth. Since saponins are usually absorbed with difficulty, persons in good health can tolerate them in large quantities taken internally in dilute solution by the mouth without deleterious effects. Toxic saponins manifest in common the property of irritating protoplasm which in larger doses they can kill. In various ways they prove that they are protoplasmic poisons. The action of saponins upon blood-corpuscles is also in harmony with this behavior. In fact R. Kobert and his collaborators have shown defibrinated blood diluted 100 times with physiological salt solution to be the best and most convenient reagent for saponin substances. Saponins cause haemolysis and the blood solution becomes laky. Agglutination and formation of methaemoglobin do not take place. The freer the blood of serum, the more pronounced is the haemolytic action of saponin substances upon blood-corpuscles. Recent investigations have shown that saponins act more vigorously upon blood-corpuscles isolated from serum because blood-serum contains cholesterol which exerts a protective influence and retards haemolysis. Quite likely haemolytic action of saponins is due to removal of cell membrane lecithin, the chief constituent of the cell-wall, from red blood-corpuscles, for lecithin-saponins are formed. Saponins also combine with cholesterol, as well as with lecithin, forming cholesterol-saponins. Once the affinities of a saponin have been satisfied by cholesterol, it ceases to act upon the lecithin of the membrane of blood-corpuscles. Consequently cholesterol prevents a saponin from causing haemolysis and so acts as an antidote for saponin substances. Ransom[1] has made the important discovery that addition of cholesterol checks the solvent action of a saponin upon blood-corpuscles. At first it was not known whether this antidotal action was due to a chemical reaction, or to adsorption, that is, to a physical process. R. Kobert,[2] as well as Madsen and Noguchi,[3] was able to dissolve cholesterol, which is insoluble in water, in an aqueous saponin solution. They assumed that this physiologically inactive solution contained a labile saponin-cholesterol compound no longer having haemolytic power. Recently Windaus[4] has definitely proved that saponin-cholesterides exist. Digitonin-cholesteride, $C_{55}H_{90}O_{29}.C_{27}H_{46}O$, crystallizes in fine needles, when a hot alcoholic solution of digitonin (1 molecule) is poured into a similar solution of cholesterol (1 molecule). This cholesteride is

[1] F. Ransom: Saponin and its Antidote. Deutsche med. Wochenschr. 1901, page 194.

[2] R. Kobert: The Saponins. Stuttgart 1904.

[3] T. Madsen and H. Noguchi: Toxins and Antitoxins, Saponin, Cholesterol. Chem. Zentralbl. 1905. I. 1265.

[4] A. Windaus: A Saponin-Cholesterol Compound. Archiv. f. exper. Pathol. u. Pharmakol. 64 (1911), 141.

formed without elimination of water. Hence in this reaction between digitonin and cholesterol we are dealing most probably with the formation of a molecular compound. Then later Yagi[1] obtained from the easily crystallizable and strongly haemolytic dioscin from Dioscorea Tokoro a crystalline cholesteride. This dioscin-cholesteride has the composition $3C_{24}H_{38}O_9.2C_{27}H_{46}O.H_2O$ (or $2H_2O$) and is formed by pouring together equi-molecular alcoholic solutions of the components. First a jelly separates and then changes in 12 hours into a crystalline mass. From alcohol it forms a colorless, micro-crystalline powder melting at 223°, very slightly soluble in water, slightly soluble in alcohol, methyl alcohol, ether and chloroform, but tolerably easily soluble in boiling alcohol and methyl alcohol. The alcoholic solution diluted with much water does not foam. The cholesteride is without haemolytic action.

Saponin solutions also dissolve white blood-corpuscles but only at higher concentrations. A physiological action characteristic of many saponins is exhibited in the stupefaction and killing of fish, even in water containing only 1:200,000 of saponin substance (R. Kobert).

Saponin substances administered internally by the mouth manifest relatively slight toxic action, at least upon dogs (Wacker[2]). Saponin, administered by the mouth daily for 6 weeks to dogs weighing about 7 kilograms, was well tolerated in doses of 0.5 gram, whereas larger quantities caused vomiting. It was tolerated better with meat than when dissolved in water or milk. Examination of the dead animal disclosed deposits of fat in the tissue of the kidneys.

In this connection mention should be made of experiments by Brandl[3] who fed various animals with Agrostemma-sapotoxin and corncockle meal prepared by him. Rabbits tolerated large quantities of these substances taken by the mouth. On the other hand, swine and dogs reacted tolerably easily but gradually became accustomed to quite considerable quantities of Agrostemma-sapotoxin. Upon the whole, relatively large quantities could be taken without injury. Consequently in animals poisoned by corncockle, disease changes of the digestive tract must have already existed. In that case a local poison having a strongly irritant action need not be introduced in large quantity to cause acute poisoning. Under such conditions small quantities of corncockle may exercise an unfavorable influence upon the digestive organs.

Rendering Saponins Non-toxic.—Saponins may be rendered non-toxic by cholesterol (see above), by treatment with hot barium hydroxide solution, and by saponification of the acetyl derivative with barium hydroxide solution or alkalies. Haemolysis serves to distinguish a toxic saponin from one that has been rendered non-toxic, since only toxic saponins act haemolytically. Saponins that have been rendered non-toxic and also glycyrrhizin cause no haemolysis whatever. The same is true of the saponin-free residues obtained by the Brunner-Rühle method of extraction (Halberkann).

[1] S. Yagi: A Saponin-Cholesterol Compound. Archiv. f. exper. Pathol. u. Pharmakol. 64 (1911), 141.

[2] L. Wacker: Action of Saponin Substances. Biochemische Zeitschr. 12 (1908), 8.

[3] J. Brandl: Toxicity of Corn-cockle Seeds. Landwirtsch. Versuchsstation 72 (1910), 326.

Variation in Strength of Haemolytic Action.—Because of differences they manifest in haemolysis, saponins introduced into the circulation exhibit different degrees of toxicity, although it is not possible to trace their toxic action exclusively to haemolysis. Then like blood-corpuscles all other cells of the organism, for example, the ganglion-cells of the brain, are also attacked by saponins. These cells require smaller doses than blood-corpuscles do and action upon them takes place more slowly. Intravenous injection of these small doses, which are quite insufficient for haemolysis, may cause death in 4–6 days. Use of plants containing saponin in fishing depends upon this action. The fish breathe through their gills and take the saponin dissolved in water direct into the circulation by which they are stupefied or killed, even in a dilution of 1:240,000.

Detection of Saponins in Effervescing Beverages

(Brunner-Rühle[1])

If acid, neutralize 100 cc. or more of the given beverage with magnesium carbonate and evaporate upon the water-bath to 100 cc. Then shake this solution vigorously with 20 grams of ammonium sulphate and 9 cc. of liquid phenol. The phenol layer containing the saponin, after separation from the aqueous layer, is shaken with 50 cc. of water, 100 cc. of ether and, if necessary, with 4 cc. of alcohol to prevent formation of emulsion. Separation of the two layers may be facilitated by gently warming the contents of the separatory funnel. The aqueous layer containing the saponin is drawn off, evaporated upon the water-bath at about 60°, and the residue dried in a vacuum desiccator. The dry residue is further purified by pouring over it 1–2 times 10 cc. portions of acetone and each time allowing it to stand for 24 hours. Acetone does not dissolve saponin and is poured off. The part of the residue remaining undissolved is tested for saponin. To detect saponin, dissolve this residue in warm water. This solution will foam strongly when shaken if saponin is present. Divide the solution into four equal portions. Evaporate one portion upon the water-bath with gentle heat and test the cold residue with concentrated sulphuric acid. Also evaporate the second portion, dilute the aqueous solution of the residue with ordinary water and introduce a small, lively fish. If a saponin is present, stupefaction and death of the fish will take place after 12–24 hours. The third portion after evaporation is taken up in 0.9 per cent. sodium chloride solution and used for the haemolytic test. If haemolysis is detected, evaporate the fourth portion and take up the residue in 0.9 per cent. sodium chloride solution. Shake this solution with an ether solution of cholesterol, taking 1 part of cholesterol to 20 parts of saponin solution and warming the mixture for several hours at 36°. The saponin solution now freed from poison by cholesterol should after evaporation of ether give a negative haemolytic test.

Notes.—According to Rühle, the guaiac-saponins prepared from wood and bark of Guaiacum officinale show, contrary to other statements, distinct haemolytic action. Neutral lead acetate precipitated from commercial saponin the acid constituent, saponic acid of Guaiac bark, and basic lead acetate the neutral saponin of Guaiac bark. Both saponins are white, pulverulent products that

[1] J. Rühle: Detection of Saponin. Zeitschr. f. Unters. von Nahrungs- u. Genussmittel 16 (1908), 165.

cause coughing and give aqueous solutions that foam strongly. In dilution 1:1000 they cause complete haemolysis and partial haemolysis in dilution 1:5000. The acid saponin has a somewhat stronger haemolytic action than the neutral body.

Coal-tar Dyestuffs.—Examination of effervescent beverages showed that they were colored with coal-tar dyes. These dyes were found in the residues tested for saponin by their haemolytic properties. In testing eight light red, yellow and green dyes, used collectively in the preparation of effervescing beverages, Rühle[1] failed to find either in glass or under the microscope any evidence of haemolysis caused by these dyes.

Dextrin.—Liquids containing dextrin are neutralized with magnesium carbonate, evaporated to 20 cc. and at once treated with 150 cc. of 96 per cent. alcohol. The mixture is allowed to settle for 0.5 hour, heated to boiling upon the water-bath, filtered hot, and the filtrate freed from alcohol after addition of a little water. The residue is finally diluted to 100 cc. with water and this solution is shaken vigorously with 20 grams of ammonium sulphate and 9 cc. of liquid phenol. In other respects the procedure is the same as described above. Effervescing lemonade powder and similar preparations containing saponin are dissolved in water to 100 cc., the solutions heated to boiling, filtered when cold and examined according to the Brunner-Rühle method.

Detection of Saponin in Oil-emulsions.[2]—Dilute 100 grams of the given oil-emulsion in a capacious beaker with the same volume of water. Stir well while adding gradually 400 cc. of 95–96 per cent. alcohol, continue stirring during 2 hours and allow to stand for 24 hours. Filter the clear liquid from the underlying fat mass through linen cloth, repeat the extraction of fat with 60–65 per cent. alcohol, and again filter. Neutralize the combined filtrates with sodium carbonate and concentrate upon the water-bath to 100 cc. Extract with ether any aromatic substances present and according to the Brunner-Rühle method add 20 grams of ammonium sulphate and shake with 9 cc. of liquid phenol. Repeat this extraction several times. Then shake the combined phenol extracts with a mixture of 100 cc. of ether, 30 cc. of water and 5 cc. of alcohol, and allow the mixture to stand for 24 hours. All saponins will have passed into the aqueous layer which is evaporated upon the water-bath. First extract the residue with acetone and test for saponin according to directions given above.

Detection of Saponin in Beverages

(Müller-Hössly[3])

This method of detecting saponin takes advantage of the property these substances have of concentrating in the foam when their solutions are shaken.

Procedure.—Neutralize in a liter cylinder 500 cc. of the beverage (1000 cc. if very little foam is formed), set the cylinder in a large funnel, and pass air through

[1] J. Rühle: Detection of Saponin, III Paper. Zeitschr. f. Unters. d. Nahrungs- u. Genussmittel 27 (1924), 192.

[2] E. Carlinfanti and P. Marzorchi: Estimation of Saponin and Saccharin in Oil Emulsions. Boll. Chim. Farm. 50 (1911), 609.

[3] E. Müller-Hössly: A Simple Test for Saponin. Mitteilung f. Lebensmitteluntersuchung u. Hygiene 8 (1917), 113.

the liquid by means of a tube extending to the bottom of the cylinder. The foam formed soon runs over, collects in the funnel, coalesces and drops into a graduate standing under the funnel. Mix 1 cc. of the liquefied foam with 1 cc. of blood solution (1 cc. of defibrinated blood + 99 cc. of physiological salt solution) and test for haemolytic action. Continue passing air through the solution until foaming has ceased. The residue in the cylinder should not have haemolytic action, if saponin has been positively detected. The sugar-content of beverages, examined by the method just described, has no influence within quite a wide range (20–200 grams of sugar per liter) upon haemolytic action. Not until the quantity falls below a certain minimal value (20 grams per liter) does haemolytic action appear even in absence of saponins and then solely because the osmotic pressure is too low. In an examination of beer 500 cc. should be taken and first neutralized with sodium carbonate until litmus paper shows a distinct blue color. The first 5 cc. should be used for the haemolytic test, for in the case of beverages and beer saponin is present in higher concentration.

Notes.—The extraordinarily high concentration of saponin in the foam constitutes the value of this method. If the operation is carried out repeatedly, using rather small containers, it is possible to reach a concentration of over 300 mg. of saponin per liter.

Detection of Saponin in Presence of Glycyrrhizin

According to Rühle,[1] the color changes given by concentrated sulphuric acid and Froehde's reagent cannot be relied upon in all cases for the positive detection of saponin, not to speak of its positive differentiation from glycyrrhizin, since residues from aqueous solutions contain many other substances, such as sugar and dyes, that interfere with these reactions. The reactions with α-naphthol and thymol, given by sugar as well, also fail. The same is true of the Brunner-Pettenkofer reaction with purified ox-gall which is a general reaction for sugar depending upon the cleavage of sugar from glucosides. Rühle has shown experimentally that by means of haemolysis saponin may be easily and positively detected in presence of considerable quantities of glycyrrhizin and that the latter cannot disguise presence of saponins.

In performing this reaction 0.9 per cent. salt solution (= Solution I), also called physiological salt solution, should be used for all solutions and dilutions. The blood solution should be defibrinated blood diluted to 1 per cent. with Solution I (= Solution II), or a suspension of red blood-corpuscles in Solution I corresponding in concentration to Solution II (= Solution III). The latter solution is used when only very little saponin is present, that is, to increase the action in consequence of removal of cholesterol from the serum. Dissolve the residue obtained by evaporating the given aqueous solution (see above) in Solution I for the saponin test. Carry out the reaction in small test-tubes holding about 10 cc. and always add for every 1 cc. of Solution II or III successively 1, 2, 3 cc. of the solution prepared for the test. Note whether the solution becomes clear. If it does, then add cholesterol to 10 cc. of the original solution showing haemolytic action and test again to ascertain whether haemolytic action has now disappeared or

[1] J. Rühle: Detection of Saponin, II Paper. Zeitschr. f. Unters. der Nahrungs- u. Genussmittel 23 (1912), 566.

not. If it has disappeared, positive proof is furnished that the given material contains saponin. In presence of very little saponin, or of substances causing turbidity such as glycyrrhizin, the reaction should be followed under the microscope.

Notes.—According to Sormani,[1] certain precautions should be observed in testing for saponin by haemolysis. All liquids examined should be isotonic and contain no substances such as acids, alkalies, alcohol and ether that may interfere with the reaction. High temperatures should be avoided and liquids should be approximately sterile. The suspensate of red blood-corpuscles used for the haemolytic test is prepared by washing defibrinated blood three times with physiological salt solution in a centrifuge. When the salt solution remains perfectly clear, 5 cc. of the centrifugalized red blood-corpuscles are diluted to 100 cc. with physiological salt solution. Such a suspensate will keep in an ice-box for 2–3 days.

To detect saponin by haemolysis in Seltzer water, beer and urine, neutralize the given liquid, expel carbon dioxide and alcohol, and finally make it isotonic with sodium citrate. Then mix such a solution with the above-mentioned suspensate of red blood-corpuscles and keep for several hours at low temperature.

In case of red wine, tannic acid compounds should first be removed by means of albumin or gelatin. The delicacy of this test in case of beer was 1:100,000; in case of Seltzer water 1:40,000.

Detection of saponin in flour, according to Rusconi,[2] requires kneading upon a tight cloth 5 grams of flour and 4 grams of 2 per cent. neutral sodium citrate solution, adding the citrate solution drop by drop. Filter 16 cc. of citrate solution obtained and add to 3 cc. of clear filtrate 1 cc. of the suspensate of red blood-corpuscles. After the un-haemolyzed blood-corpuscles have settled, if the supernatant liquid appears red and transparent, saponin is present. To detect saponin in bread (detection of Agrostemma), heat 10 grams of powdered bread to boiling three times, using each time 5 cc. of 95 per cent. alcohol, 5 cc. of chloroform and pouring quickly through a dry filter. Evaporate the filtrates upon the water-bath, dissolve the residue in physiological salt solution, filtering if necessary. Otherwise the procedure is the same as described above.

Detection of Saponins

(Rosenthaler[3])

Some saponins such as guaiac-saponin and saponin from Bulnesia Sarmienti exhibit slight or no haemolytic action at all. In such cases the method of Rühle is more or less of a failure. It is also possible that regenerated saponins without haemolytic action are commercial products. Rosenthaler does not isolate pure saponins but their water-soluble products of hydrolysis designated as pro-sapogenins.

Procedure.—Add to the given liquid sufficient hydrochloric acid to bring the percentage up to about 2.5 per cent. First filter the solution, if necessary, and

[1] C. Sormani: Detection of Saponin in Beverages and Foodstuffs. Zeitschr. f. Unters. d. Nahrungs-u. Genussmittel 23 (1912), 561.

[2] Rusconi: Giornale di Farmacologia sperimentale 1912.

[3] L. Rosenthaler: Detection of Saponins. Zeitschr. f. Unters. d. Nahrungs- u. Genussmittel 25 (1913), 154.

then heat until hydrolysis is complete, that is, until the liquid no longer foams when shaken. Allow the solution to cool without filtering and shake while still warm with acetic ether, using 5 cc. of this solvent for every 100 cc. of liquid. Break up any emulsion that may form by adding a little alcohol. Wash the clear acetic ether solution with a little water, using 5–10 cc. each time, until Cl′-ion has disappeared. Then evaporate the solution, if it has only a slight color, otherwise discharge the color by means of animal charcoal. Use the residue from the acetic ether solution to make the color test with concentrated sulphuric acid. A positive result given by this test is accepted as conclusive. Another portion of residue is dissolved in sodium carbonate solution and the solution shaken to see whether it still foams. Pro-sapogenin dissolves very slightly in water even at boiling temperature but is soluble in acetic ether, ethyl and methyl alcohol, acetic acid and also slightly soluble in chloroform. It possesses acid properties.

To detect saponin in beer, first heat 100 cc. of the latter under a reflux with 100 cc. of 95 per cent. alcohol until a flocculent precipitate appears. Filter this precipitate and use the filtrate, after distilling off alcohol, for hydrolysis as directed above. Employing this method, Rosenthaler was able in every instance to detect saponin. Presence of glycyrrhizin did not interfere with the detection of saponin!

According to Sagel,[1] detection of saponin by the color reaction with concentrated sulphuric acid may actually be hastened and intensified by addition of acetic anhydride. Rub a small quantity of the given product upon a watch-glass with 2 drops of acetic anhydride and then allow 1 drop of concentrated sulphuric acid to run down from the margin. In presence of saponin, a distinct light red color like that of a dilute fuchsine solution immediately appears.

Detection of Corn-cockle in Flour

This test is due to presence in corn-cockle of the saponin githagin. Heat 500 grams of the flour with 1 liter of 15 per cent. alcohol, filter hot, evaporate the filtrate to smaller volume, and separate saponin by addition of absolute alcohol and some ether. After the mixture has stood for 12–24 hours, any precipitate that has formed is collected upon a filter, dried at 100° to coagulate admixed protein substances, then dissolved in a little cold water, and saponin again precipitated from the filtered solution by absolute alcohol and ether. Saponin that now separates is detected by its sharp, harsh taste, foaming of the aqueous solution, behavior with concentrated sulphuric acid (githagin is soluble with yellow color gradually changing to purplish red), and haemolytic action. Further tests that may be employed for detection of githagin are the reducing action upon ammoniacal silver nitrate solution and Fehling's solution after previous boiling with hydrochloric acid.

Microscopic examination of the flour will also reveal presence of corn-cockle by the characteristic structure of the seed envelopes.

[1] K. Sagel: Detection of Saponin. Pharmazeutische Zentralhalle 55 (1914), 268.

Quantitative Estimation of Saponins in Plant Powders

(Korsakow[1])

This method is based upon the following observations: 1. Saponins form unstable compounds with magnesium oxide. 2. Saponins are soluble in boiling 80 per cent. alcohol but insoluble in an ether-alcohol mixture. 3. Saponin is not weighed as such but as sapogenin formed by hydrolysis of saponin.

Procedure.—Make repeated extractions of the dry plant powder with boiling 60 per cent. alcohol, filter the extract, distil off the alcohol, and evaporate the residue together with a little magnesium oxide to dryness upon the water-bath. Powder the dry residue, exhaust it in a Soxhlet with boiling 80 per cent. alcohol, filter, and precipitate saponin from the filtrate with ether. Dissolve the precipitate in 3 per cent. sulphuric acid, heat the solution for 1 hour in sealed tube to 105°, wash separated sapogenin upon the filter with water to neutral reaction, dissolve in absolute alcohol, expel alcohol, bring the precipitate to constant weight in vacuum desiccator, and weigh.

SOLANINE AND SOLANIDINE

Solanine, $C_{52}H_{93}NO_{12}$, at the same time an alkaloid and glucoside (glucoalkaloid) occurs in the potato plant (Solanum tuberosum) and in other Solanaceae such as Solanum nigrum, Solanum dulcamara and Solanum lycopersicum (tomato). It has been found also in Scopoliaceae, such as Scopolia orientalis and Scopolia atropoides. Solanine is not uniformly distributed in all parts of the potato plant but is most abundant in the berry-like fruit and in the chlorophyll-free sprouts appearing in the spring upon potatoes that lie in a cellar. Schmiedeberg and Meyer found 0.024 gram of solanine per kilogram of peeled potatoes in January and February but 0.044 gram in unpeeled potatoes. Potato peelings gave 0.71 gram of solanine per kilogram and potato sprouts 1 cm. long even 5.0 grams. New potatoes that have not wholly matured contain 5–6 times as much solanine as do those that have matured. The same is also true of the black spots upon very old potatoes. Wide-spread poisonings have repeatedly occurred as the result of eating such potatoes. R. Weil claims to have shown that two of the bacteria found in the black spots upon potatoes have the power of producing solanine. Rather serious illness may appear in man from taking 0.3–0.4 gram of solanine with potatoes.

Properties.—Solanine crystallizes in white needles having a bitter taste and melting at 244°. Even boiling water dissolves only a little of this alkaloid (about 1:8000). It is soluble in 500 parts of cold and 125 parts of boiling alcohol; and in about 4000 parts of ether. These solutions are faintly alkaline. Hot saturated solutions of solanine in alcohol and amyl alcohol gelatinize upon cooling. Ether, chloroform and benzene do not extract solanine either from acid or alkaline solution. But hot amyl alcohol extracts solanine from acid solution and from solutions alkaline with sodium hydroxide or ammonia. Solanine is a weak base, readily dissolving in acids, such as acetic acid, forming crystalline salts. Dilute hydrochloric or sulphuric acid hydrolyzes solanine to solanidine,

[1] M. Korsakow: Investigations of the Methods of Estimating Saponins. Comptes rend. de l'Acad. des Sciences 155 (1913), 844.

$C_{40}H_{61}NO_2$, galactose and rhamnose. Hydrolysis is very slow in the cold but rapid upon heating. The hydrochloride or sulphate of solanidine separates as a difficultly soluble, crystalline powder. A good yield of solanidine is obtained, according to Wittmann, by heating solanine under a reflux with 10 times the quantity of 2 per cent. sulphuric acid, until the liquid is yellowish and the filtrate upon further boiling no longer deposits solanidine sulphate. Solanidine, precipitated from its sulphate with ammonia and recrystallized from ether, forms colorless, silky needles, melting at 207° and dissolving with difficulty in water but readily in ether or hot alcohol. Solanidine is a stronger base than solanine and the salts it forms with acids are usually crystalline and difficultly soluble in water.

Toxic Action.—Solanine and solanidine are highly toxic substances having an action similar to that of saponin substances (see above). Solanine taken internally is usually very imperfectly absorbed. As a glucoside its action is local and as a saponin-like substance strongly haemolytic, rendering the blood laky. A solanine solution even in dilution of 1 : 8300 causes complete haemolysis. Internal administration of solanine usually produces emesis and larger doses cause gastro-enteritis (gastro-intestinal catarrh). The latter also follows intravenous and subcutaneous injection of doses not rapidly fatal. At the same time a haemoglobinuria may appear. In the case of wide-spread poisoning affecting 673 soldiers in Strassburg, evidences of intoxication were headache, severe colicky pains in stomach and abdomen, vomiting, diarrhoea, general depression, and some mental confusion. In individual cases face and lips were cyanotic, pulse quickened, pupils enlarged, and temperature elevated to 39.5°. Only in isolated cases did convulsions appear. In rare cases it terminated in syncope and collapse.

Detection of Solanine and Solanidine

Since very dilute mineral acids hydrolyze solanine, these acids cannot be used to detect this alkaloid. E. Schmidt[1] suggests the following procedure. Extract the material with cold water containing tartaric acid. Neutralize the filtered extract with calcined magnesia and evaporate to dryness upon the water-bath. Extract the residue with alcohol and filter hot. If the quantity of solanine is not too small, the alcoholic extract gelatinizes upon cooling. Otherwise, evaporate the alcoholic solution and examine the residue for solanine. R. Kobert extracts solanine from alkaline solution with isobutyl alcohol. Phospho-molybdic acid is the only general reagent giving a precipitate with a solanine solution and that is yellow. But solanidine, that is, a solanine solution that has been boiled with excess of hydrochloric acid, being a stronger base, gives precipitates with most of the other general reagents.

Reactions of Solanine and Solanidine

1. Selenic-Sulphuric Acid[2] Test.—Solanine and solanidine dissolve in this reagent giving a raspberry-red color. Gentle heat favors appearance of the

[1] Pharm. Chem., Organ. Theil.

[2] 0.3 gram of sodium selenate, $Na_2SeO_4.10H_2O$, 8 cc. of water, and 6 cc. of concentrated sulphuric acid.

reaction. Sensitiveness in case of solanine is 0.025 mg.; in case of solanidine 0.01 mg. in 1 cc.

2. **Vanadic-Sulphuric Acid[1] Test.**—Solutions of solanine and solanidine in this reagent are orange-yellow, soon becoming red and finally blue-violet Solanine may be first dissolved in sulphuric acid and a drop of the reagent added to this solution.

3. **Ethyl-Sulphuric Acid[2] Test.**—Solutions of solanine and solanidine in this reagent are red. An alcoholic solution of solanine, carefully added to concentrated sulphuric acid as an upper layer, produces a red zone where the two liquids meet and below that a yellow zone.

4. **Sulphuric Acid Test.**—A solution of solanine in concentrated sulphuric acid is orange but becomes brownish red on longer standing or gentle warming. Red streaks appear, if bromine water is added drop by drop to a solution of solanine in concentrated sulphuric acid.

5. **Froehde's Test.**—A solution of solanine in this reagent is first yellowish red, then evanescent cherry-red and finally red-brown.

Estimation of Solanine in Potatoes

1. **Method of O. Schmiedeberg and G. Meyer.**—Mix 500 grams of finely grated potatoes with water and press out the liquid. Decant liquid from the deposit of starch. Again mix the starch with water and decant the latter when the starch has settled. Neutralize the entire liquid with ammonia and evaporate to the consistency of an extract. In the meantime mix the press-cake with several times its volume of boiling alcohol. Press out the alcohol completely after several hours. Make two such extractions. Filter the combined alcoholic extracts and wash the residue (starch) upon the filter with alcohol. The aqueous liquid from the potatoes contains very little solanine. To isolate this small quantity, use the alcoholic filtrate to extract the residue from the aqueous extract and again filter. Wash the insoluble part with hot alcohol. The alcoholic filtrate after half an hour usually deposits some crystals of asparagine.[3] Separate the supernatant liquid from these crystals and evaporate upon the water-bath to the consistency of an extract. Dissolve the residue in water containing sulphuric acid, filter and wash. Warm the clear liquid very gently, saturate with ammonia, and set aside for a day. Solanine appears in small crystals. Collect the deposit upon a weighed filter, wash first with water, then with ether, dry at 100° and weigh.

[1] Solution of 0.1 gram of ammonium vanadate, $(H_4N)VO_3$, in 100 grams of concentrated sulphuric acid.

[2] 9 cc. of absolute alcohol and 6 cc. of concentrated sulphuric acid.

[3] Asparagine is the amide of aspartic acid, monamino-succinic acid amide,

$$HOOC.CH(NH_2).CH_2.CO.NH_2$$

It forms shining, rhombic crystals that dissolve rather easily in hot water but less easily in alcohol or ether. Of the three asparagines, laevo-, dextro- and inactive asparagine, the first is widespread in the plant kingdom in seeds.

2. Method of von Morgenstern.[1]—Express as much liquid as possible from 200 grams of finely grated potatoes by means of a press. Make two separate extractions of press-cake with water and express the liquid thoroughly each time. Precipitate protein substances from the combined liquid by adding 0.5 cc. of acetic acid and warming for 1 hour upon the water-bath. Filter, evaporate the filtrate to a syrup, stir, and gradually add hot 96 per cent. alcohol until cloudiness ceases.[2] Decant the solution after 12 hours and extract the residue containing sugars and dextrins twice with hot alcohol. Evaporate the combined alcoholic extracts upon the water-bath, warm the residue with some water containing acetic acid, and filter. Heat the filtrate to boiling and add ammonia drop by drop to precipitate solanine. After standing for 5 minutes upon the water-bath, the base separates in flocks that are easily filtered. Wash the precipitate with water containing ammonia, dissolve in boiling alcohol, and treat this solution as follows. Evaporate upon the water-bath and dissolve the residue in water containing acetic acid. Filter, heat the filtrate to boiling, and precipitate solanine with ammonia. Collect the pure white flocks of solanine from this second precipitation upon a filter that has been dried at 90° and weighed. Wash with 2 per cent. ammonia and dry at 90° to constant weight.

Notes.—v. Morgenstern obtained upon the average by this method 0.0125 per cent. of solanine in table potatoes and 0.0958 per cent. in those used as forage. The yield of solanine from yellow tubers upon the average was less than from red. Tubers grown upon sandy soil were richer in solanine than were those from humus soil. Moisture and abundance of humus appear to diminish the quantity of solanine. Nitrogenous fertilizer increased the quantity of solanine, potash fertilizer lowered it, and phosphate fertilizer appeared to have little effect. There was less solanine in large than in small potatoes of the same variety. Solanine first appears to increase during the process of germination. Passing into sprouts, without wholly disappearing from the tubers, solanine increases with the growth of the plant. As growth advances, distribution of solanine in the different parts of the plant is indication of a tendency on the part of the plant to withdraw solanine from the older sprouts and spread it throughout the young organs. Consequently solanine may serve first of all as the natural protector of the plant, especially of the growing parts. Putrefaction diminished the solanine content hardly at all. Rust, moist rot and rhizoktonia scab had no influence upon solanine content.

EXAMINATION OF NATURAL PRODUCTS

ERGOT

Officinal ergot (Secale cornutum) is the sclerotium (compact mycelium or spawn) of Claviceps purpurea collected from rye shortly before the fruiting

[1] F. v. Morgenstern: Solanine in Potatoes used as Food and Fodder and Influence of the Soil upon Formation of Solanine in the Potato Plant. Landw. Versuchsstation 65 (1907), 301.

[2] Other parts of the potato plant, that is, those that can be dried at 100° and reduced to powder, are extracted by heating to boiling with water containing acetic acid and filtering.

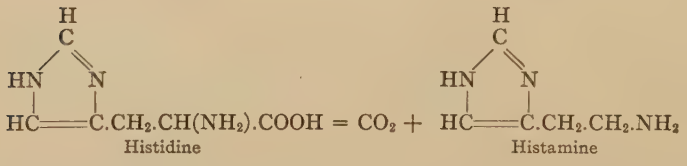
period and dried at gentle heat. Ergot is commonly known as an abortifacient and intoxications have occurred from its use. Consequently examinations for legal purposes may require detection in powders and other mixtures. Our knowledge of the constituents of ergot is still very defective notwithstanding several exhaustive investigations. Ergot alkaloids, namely, ergotine, ergotinine, cornutine, picro-sclerotine, were described long ago. But, with the possible exception of ergotine (Tanret, C. C. Keller), the preparations were not entirely pure. Ergot contains in addition to alkaloids other peculiar chemical substances which have received but little attention. They have not the characteristic physiological action of ergot but, like the pigment sclererythrin, are useful for purposes of identification. Among these substances belong sphacelic acid and sclerotic acid, according to R. Kobert, a very poisonous resin having acid properties.

Alkaloids.—The most recent[1] researches upon ergot mention as well characterized bases, ergotinine, $C_{35}H_{39}N_5O_5$, and hydro-ergotinine, $C_{35}H_{41}N_5O_6$. Barger calls the latter ergotoxine. Ergotinine crystallizes from alcohol in long needles melting at about 229° when heated rapidly. This componud dissolves in 52 parts of boiling alcohol; in 1.1 parts of ether; and is readily soluble in chloroform. Crystalline salts of this base have not yet been prepared. Ergotoxine (= hydrate of ergotinine), obtained as a crystalline phosphate from ergotinine mother-liquors by means of alcohol and phosphoric acid, is a white powder softening at 155° and melting at 162–164°. Though freely soluble in alcohol, it dissolves but slightly in ether. As a rule the salts of ergotoxine crystallize well.[2] By preparing a cold methyl alcohol solution of ergotoxine and boiling this solution for several hours under a reflux, F. Kraft converted this substance completely into ergotinine. On the other hand, ergotinine in dilute acetic acid solution passes back almost entirely into hydro-ergotinine within 10 days. As an indication of purity, a solution of hydro-ergotinine in 2 parts of cold methyl alcohol after several days standing should not deposit crystals (ergotinine) nor become green. Ergotoxine and ergotinine may be extracted by chloroform and ether from neutral and even from faintly acid solutions. Therefore both alkaloids must be only weak bases.

Histamine and Tyramine.—Progressive decomposition of ergot and its extracts produces with cleavage of carbon dioxide from the amino-acids present, namely, tyrosine and histidine, the bases p-oxy-phenyl-ethyl-amine (= tyramine) and β-imidazolyl-ethyl-amine (= histamine):

$$HO.C_6H_4.CH_2.CH(NH_2).COOH = CO_2 + HO.C_6H_4.CH_2.CH_2.NH_2$$

Tyrosine — Tyramine



Histidine — Histamine

[1] F. Kraft: Ergot. Archiv. d. Pharm. 244 (1906), 336; G. Barger: Journ. chem. Soc. London 91, 337.

[2] Bio-Chem. Journ. 2, 240.

Tyramine strongly contracts blood-vessels. On the other hand, histamine produces enlargement of blood-vessels and diminished blood-pressure, also contraction of many smooth muscles, especially strongly those of the uterus.

Ergotoxine and β-imidazolyl-ethyl-amine (= histamine) thus far are the only pure substances prepared from ergot that exert an action upon the movements of the uterus. Tyramine crystallizes from alcohol in hexagonal leaflets melting at 161°, dissolves in 10 parts of boiling alcohol, is less soluble in water, and gives a red coloration with Millon's reagent. The picrate consists of prisms from water melting at 200°. Barger obtained from ergot 0.01–0.1 per cent. of tyramine. Choline, $(CH_3)_3N(CH_2.CH_2.OH)(OH)$, is also a constituent of ergot.

Reactions of Ergotoxine and Ergotinine

1. Both alkaloids dissolve in alcohol with blue fluorescence.

2. The solution of these alkaloids in concentrated sulphuric acid is light yellow, changing after several hours to very stable violet-blue that does not fade out for days.

3. A trace of ferric chloride solution, added to the concentrated sulphuric acid solution of these alkaloids, produces first an orange-red and then a deep red color; gradually extending from the margin inward, a bluish to bluish green coloration appears.

Physiological Action of These Alkaloids.—Ergotinine and hydro-ergotinine, according to Jaquet, produce convulsions and gangrene. They are not, however, the cause of the specific uterine contraction characteristic of ergot. Keller's cornutine, according to Kraft, is identical with ergotinine, according to Barger and Dale,[1] with ergotinine impure from ergotoxine (hydro-ergotinine). The English investigators believe that the physiological effects observed with ergotinine are due to adhering ergotoxine. The latter is readily formed when difficultly soluble ergotinine is brought into solution by means of glacial acetic acid, phosphoric acid, or a little sodium hydroxide solution. Ergotoxine, according to Barger and Dale, produces the effects typical of ergot, causing powerful contraction of the uterus and later abortion.

Schlererythrin.—This is the pigment of the outer coat of ergot. E. Schmidt gives the following directions for its isolation. Extract freshly powdered ergot with ether to remove fat. Then moisten the powder with water containing tartaric acid, dry and extract with 95 per cent. alcohol. Filter and distil the alcohol. Extract the residue with ether. This solvent now dissolves sclererythrin which may be precipitated by means of petroleum ether.

Sclererythrin is an amorphous red powder that can be sublimed. It is insoluble in water but soluble in absolute alcohol and glacial acetic acid. This substance behaves like an acid, dissolving in caustic alkalies, ammonia, and alkaline carbonate and bicarbonate solutions with red or red-violet color. Owing to presence of sclererythrin, ether, if shaken with powdered ergot moistened with tartaric acid solution, becomes red. If such an ether solution of sclererythrin is shaken with sodium hydroxide solution, the pigment dissolves in the latter which then becomes red. Solutions of this pigment show characteristic absorption-bands in the spectrum.

[1] G. Barger and F. H. Carr: Alkaloids of Ergot. Proc. Chem. Soc. 23, 27.

Detection of Sclererythrin

(Schaer[1])

Selected particles of the material that might come from ergot are stirred with a little 60 per cent. chloral hydrate solution which after some time is poured through a small filter. In presence of scelrerythrin the solution has a light cherry-red color. A drop of this solution upon thick filter paper leaves upon evaporation a permanent light red spot with a brownish margin. This spot moistened with alcoholic ammonia becomes violet.

The rest of the chloral hydrate solution, after acidification with 1–2 drops of dilute sulphuric acid, is extracted with ether. The pigment passes into the ether producing a rose color. Then sclererythrin may also be detected spectroscopically.

When not possible to select particles of ergot, isolation of sclererythrin direct from the material may be undertaken. In that case, extract for 12 hours at 40° with 80 per cent. alcohol. Then express the liquid and filter. Concentrate the filtrate *in vacuo*, take up with acidified water containing alcohol and extract with ether. In presence of sclererythrin the ether solution has a reddish color and upon spectroscopic examination exhibits the properties given below.

Detection of Ergot in Flour, Bread and Powders.—This examination usually consists in undertaking to detect by chemical and physical means the red pigment sclererythrin characteristic of ergot. The property possessed by this substance of passing from ether into a solution of an alkaline hydroxide or bicarbonate is especially valuable for purposes of identification.

According to Hoffmann, 10 grams of flour are well worked in a mortar with a few drops of sodium hydroxide solution for the purpose of first loosening up the material. It is then treated drop by drop with dilute sulphuric acid until a distinct acid reaction is given after kneading. Then it is rubbed gradually with 20 grams of ether, rinsed into a shaking flask, and agitated for several hours in a shaking-machine. The ether extract after settling is filtered and shaken with 10–15 drops of cold saturated sodium bicarbonate solution. The latter takes the pigment from ether and is itself colored violet-red. The positive recognition of sclererythrin in the violet-red sodium bicarbonate is undertaken by means of the spectroscope. The alkaline sodium bicarbonate solution of sclererythrin exhibits three absorption-bands, the one most marked being to the right of D in the yellow. The other two bands are weaker, one being somewhat to the right of E and the third to the right of F in the green. Then acidify the solution with sulphuric acid and extract the pigment with ether. This ether solution shows only two absorption-bands, one in the green E and F but nearer to E, and a second broader band in the blue midway between F and G.

The spectroscopic examination should be made immediately after preparing the solution of the pigment, for the solutions are not very permanent. Have the layer of liquid of such depth that the colors stand out strongly. Then the absorption-bands are distinctly visible, otherwise the result in uncertain.

Detection of Choline.—Ergot powder, warmed with dilute potassium hydroxide solution, gives the characteristic odor of trimethylamine, $(CH_3)_3N$, due to decomposition of choline in ergot. The so-called corn smut (Ustilago Maidis), said to

[1] E. Schaer: Archiv d. Pharmaz. 228 (1890), 370.

cause effects similar to those of ergot, also gives the trimethylamine odor when warmed with potassium hydroxide solution, for it contains appreciable quantities of choline. Occasionally flour that does not contain ergot may give an odor when heated with potassium hydroxide solution but it cannot be mistaken for the odor of trimethylamine.

Detection and Quantitative Estimation of Ergotinine and Ergotoxine (Keller).— Dry finely powdered ergot over lime, place 25 grams in a Soxhlet tube and completely extract fat with petroleum ether. Dry the powder at gentle heat, add 100 grams of ether and after 10 minutes shake well with milk of magnesia (1 gram of MgO and 20 cc. of water). Shake repeatedly during an hour and then pass 80 grams of the ether solution (= 20 grams of ergot) through a covered folded filter into a separating funnel. Shake the ether in succession with 25, 15, 10 and 5 cc. of 0.5 per cent. hydrochloric acid. Pour the hydrochloric acid extracts, now containing the ergot alkaloids, through a small moistened filter.[1] Add ammonia until alkaline and extract the solution twice with about half its volume of ether. Let the ether extract settle in a dry flask, then filter into a dry weighed flask and wash the filter with a little ether. Distil the ether and dry flask and residue at 100° to constant weight. Good German ergot contains 0.13–0.16 per cent. and Russian ergot 0.22–0.25 per cent. of alkaloid.

To detect ergot alkaloid qualitatively, proceed as follows:

(a) Dissolve a part of the residue in 1 cc. of concentrated sulphuric acid and add a trace of ferric chloride solution. The mixture is orange-red and becomes at once deep red but the margin appears bluish to bluish green.

(b) Dissolve a part of the residue in about 4 cc. of glacial acetic acid and add a trace of ferric chloride solution. Cautiously add this mixture to concentrated sulphuric acid as an upper layer. If ergotinine is present, a brilliant violet color appears where the two liquids meet.

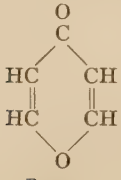
"Tenosin Bayer" contains the two active constituents of Secale cornutum, β-imidazolyl-ethyl amine and p-oxy-phenyl-ethyl amine, in chemically pure condition and in such proportion by weight that the action of β-imidazolyl-ethyl amine in decreasing blood-pressure is balanced by the action of p-oxy-phenyl-ethyl amine in increasing blood-pressure. Tenosin is free from ergotoxine, an alkaloid producing Secale gangrene. Tenosin is said to be a valuable substitute for Secale cornutum.

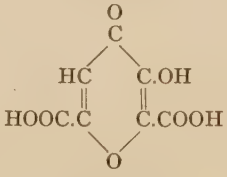
OPIUM

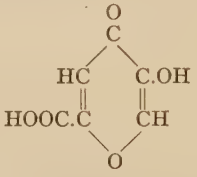
Opium and its two pharmaceutical preparations, extract and tincture of opium, contain, in addition to the alkaloids, two compounds characteristic of opium, namely, meconic acid and neutral meconine. Upon the average, opium contains 4 per cent. of meconic acid and 0.3 per cent. of meconine. Since poisoning from opium, especially in the case of suicides, occurs rather frequently as a result of taking opium itself as well as its pharmaceutical preparations, meconic acid and meconine are of significance to the forensic chemist in definitely establishing opium poisoning.

[1] In case these hydrochloric acid extracts do not filter clear, they should be clarified by being shaken with a little talcum powder, that has previously been treated with hydrochloric acid and thoroughly washed with water, and filtered several times.

Meconic Acid.—This compound is oxy-pyrone-dicarboxylic acid, $C_7H_4O_7.3H_2O$. It crystallizes in rhombic plates and leaflets that lose crystal water at 100° and melt at 150°. It dissolves with difficulty in cold water, alcohol and ether; more easily in boiling water and in hot alcohol; and very readily in methyl alcohol and acetic ether. When boiled with water, or better with dilute acid, meconic acid loses a molecule of carbon dioxide and is converted into comenic acid:


Pyrone


Meconic acid

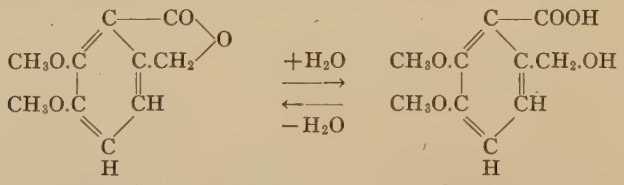

Comenic acid

Quite recently Thoms[1] has obtained meconic acid synthetically.

Detection of Meconic Acid

Extract the given material with alcohol containing a few drops of hydrochloric acid. Filter and evaporate the filtrate upon the water-bath almost to dryness. Dissolve the residue in water, filter, heat the filtrate to boiling and shake with excess of calcined magnesium oxide. The solution contains magnesium meconate. Filter the solution while still hot, evaporate to smaller volume, and acidify faintly with dilute hydrochloric acid. Add a few drops of ferric chloride solution. A blood-red to brown-red color appears, if meconic acid is present. Neither addition of dilute hydrochloric acid nor warming should discharge this red color, in which respects it differs from ferric acetate. This color differs from that caused by sulphocyanic acid in not being affected by addition of gold chloride. By this method, the author succeeded in getting a distinct test for meconic acid in an aqueous extract from 0.05 gram of opium. The test was also given by urine that had stood for two years and had been taken from a person poisoned by tincture of opium.

Meconine.—This compound is the internal anhydride (lactone) of meconinic acid and has the formula $C_{10}H_{10}O_4$. It crystallizes in colorless prisms having a neutral reaction, melts at 102–103°, and sublimes without decomposition. It dissolves with considerable difficulty in cold water (1:700), fairly easily in boiling water (1:22), and readily in alcohol, ether, benzene and chloroform. As the lactone of a γ-oxy-acid, that is, of meconinic acid, meconine is easily converted by caustic alkalies into soluble salts of this acid. Free meconinic acid, however, does not exist as such but upon separation from its salts at once forms water and its anhydride meconine:


Meconine — Meconinic acid

[1] H. Thoms and R. Pietrulla: Synthesis and Constitution of Meconic A Ber. d. Deutsch. Pharm. Ges. 31 (1921), 1.

Detection of Meconine

Following the usual procedure of the Stas-Otto process, substituting dilute sulphuric for tartaric acid, prepare an extract of the material. Extract the sulphuric acid solution thus obtained with benzene which dissolves meconine. Evaporation of the solvent often leaves the latter as a crystalline residue. Meconine if present will dissolve in concentrated sulphuric acid with green color changing to red in the course of two days. Very careful warming of this sulphuric acid solution, that is greenish or has become red, will produce an emerald-green color becoming blue-violet and finally red.

PTOMAINES

Ptomaines are basic substances containing nitrogen and may be toxic or non-toxic. They are produced during putrefaction of cadavers under the influence of bacteria. They are to be regarded to some extent as products of bacterial metabolism and are nearly always present in cadavers, especially in those parts that are in an advanced state of putrefaction. Many ptomaines closely resemble alkaloids. Like alkaloids they give precipitates with general reagents, and certain ptomaines resemble well-defined alkaloids even with special reagents. Hence ptomaines are of very great importance in forensic chemistry, since their presence may easily lead to mistakes and false conclusions. These putrefactive products also resemble vegetable bases in their behavior with solvents. Ether extracts some of them from acid solution and others from alkaline solution, whereas certain ptomaines are removed from alkaline solution only by amyl alcohol or chloroform. Most of the ptomaines are strong reducing agents, for example, they will immediately convert potassium ferricyanide into ferrocyanide. Consequently, they give the Prussian blue test with a dilute mixture of solutions of ferric chloride and potassium ferricyanide. Many of the alkaloids like morphine resemble the ptomaines in this respect.

The resemblance of a ptomaine to a definite vegetable base is frequently confined to some one reaction, and never extends to all the reactions characteristic of the particular alkaloid. In a legal-chemical investigation no precaution, that guards against mistaking a ptomaine for a vegetable base, should be omitted. It is an invariable rule to make every test characteristic of the suspected alkaloid, and not to be satisfied with possibly one positive test. A determination of the physiological action of the substance should supplement the chemical examination. A ptomaine may resemble a vegetable

base chemically, and yet the two substances may differ very decidedly in physiological action. Thus far, ptomaines have been found that show certain resemblances to aconitine, coniine, nicotine, strychnine, codeine, veratrine, delphinine, atropine, hyoscyamine, morphine and narceine. Selmi has described a putrefactive product that resembles morphine. Ether failed to extract it either from acid or alkaline solution, whereas amyl alcohol removed it with ease from an alkaline or ammoniacal solution. It liberated iodine from iodic acid but failed to give the tests characteristic of morphine alone, namely, Husemann's, Pellagri's and the ferric chloride test!

The object in such cases must be to get a result about which there can be no doubt. Every possible means should be used to isolate the alkaloid in a perfectly pure state. When this can be accomplished, the nature of the poison can always be established beyond question.

Isolation of Ptomaines

(Brieger[1])

Bring the mass of the flesh or organ to as fine a state of division as possible and heat to boiling for a few minutes with water containing dilute hydrochloric acid. During boiling, a weak acid reaction should persist. Filter and evaporate the filtrate first upon a gas-plate and later upon the water-bath to a thick syrup. Evaporation preferably should be carried on *in vacuo*, since this procedure prevents fetid vapors from becoming a nuisance. Dissolve the thick syrupy liquid thus obtained in 96 per cent. alcohol. Filter and treat the filtrate with warm alcoholic lead acetate solution. Again filter, evaporate the filtrate to a syrup, and extract with 96 per cent. alcohol. Dissolve in water the residue left after evaporating alcohol and remove dissolved lead by means of hydrogen sulphide. Filter and concentrate the filtrate to a syrup after adding a little hydrochloric acid. Again dissolve the syrup in alcohol, filter, and add to the filtrate sufficient alcoholic solution of mercuric chloride for complete precipitation. Extract the filtered and washed precipitate (N) with water and allow what is dissolved in the filtrate to crystallize fractionally. Differences in solubility of the mercury double compounds sometimes make possible separation of individual ptomaines. Evaporate the alcoholic filtrate (F), having first removed mercury by means of hydrogen sulphide. Add sodium carbonate, leaving the reaction faintly acid, evaporate, and extract repeatedly with alcohol to get rid of inorganic products as completely as possible. Expel alcohol from these extracts, dissolve the residue in water, and neutralize the solution with sodium carbonate. Acidify with nitric acid and precipitate with phospho-molybdic acid. Decompose the precipitate upon the water-bath with neutral lead acetate, remove lead from the filtered solution by means of hydrogen sulphide, filter, evaporate to a syrup, and treat with alcohol. By this procedure various ptomaines are removed as hydro-

[1] L. Brieger: Researches upon Ptomaines, Part III, page 19. Berlin 1886.

chlorides. To effect separation of individual ptomaines, it is advisable to convert them into double salts, since the hydrochlorides themselves are almost never obtained in very pure condition. To separate ptomaines, such reagents as chloro-platinates, chlor-aurates and picrates are used. The precipitates obtained are then purified by recrystallization. Differences in solubility of these salts in a definite solvent often make possible the preparation of individual ptomaines in a state of purity. At the same time the fact should not be overlooked that many gold double salts are decomposed when warmed in aqueous solution. Addition of hydrochloric acid, however, will easily prevent such a change from taking place. Determination of melting-point after each recrystallization will show when the given salt is pure, for it will remain constant when that point is reached. Elementary analysis of the given salt is an additional means of identifying the ptomaine.

To obtain pure ptomaine hydrochlorides, chloro-platinates, chlor-aurates and mercury double salts should be decomposed by means of hydrogen sulphide. Picrates on the other hand should be dissolved in water, the solution acidified with hydrochloric acid, and picric acid removed by repeated extraction with ether. Purification of individual ptomaines is quite difficult because of a nitrogenous, non-toxic, amorphous protein substance dissolved by most solvents and removed only by careful precipitation with neutral alcoholic lead acetate. In getting rid of this impurity by this method, excess of lead acetate should be avoided, otherwise this particular protein substance will be redissolved. Once this objectionable protein substance, which forms an amorphous compound with platinic chloride and has very strong reducing properties, is removed, hydrochlorides, chlor-aurates and chloro-platinates of the different ptomaines usually crystallize without further difficulty.

I. Ptomaines from Human Cadavers

Employing the method described above, Brieger investigated quantities as large as 100 grams of such human internal organs as heart, liver, lungs, spleen, kidneys, etc., and succeeded in isolating the following ptomaines:

(a) From the precipitate produced by mercuric chloride (N): a considerable quantity of the double salts of cadaverine and putrescine with very little mydatoxine. After these had been treated with hydrogen sulphide to remove mercury, the solution of the hydrochlorides was evaporated and the salts separated by means of alcohol.

Cadaverine Hydrochloride.—This compound is pentamethylene-diamine hydrochloride and has the formula $HCl.H_2N.(CH_2)_5.NH_2.HCl$. It is quite easily soluble.

Putrescine Hydrochloride.—This compound is tetramethylene-diamine hydrochloride and has the formula $HCl.H_2N.(CH_2)_4.NH_2.HCl$. Unlike cadaverine it remains for the most part undissolved.

To obtain these two ptomaines absolutely pure, it was found necessary to have recourse to difference in behavior of their chlor-aurates and mercuric chloride double salts toward water. Putrescine chlor-aurate is rather difficultly soluble in cold water, whereas the same salt of cadaverine is very easily soluble. On the other hand, mercuric chloride double salts behave in the reverse manner. Attendant impurities remain dissolved in the mother-liquors. In addition to

the two non-toxic bases, cadaverine and putrescine, Brieger also obtained from parts of putrefied human cadavers in smaller quantity a toxic base having the composition $C_6H_{13}NO_2$. This he called mydatoxine. It occurs in the mother-liquors of the mercuric chloride double salt of cadaverine and putrescine. After mercury had been precipitated by means of hydrogen sulphide, it was isolated as chloro-platinate and obtained pure by repeatedly crystallizing from absolute alcohol containing hydrochloric acid. This salt melts at 193° with decomposition and has the formula $(C_6H_{13}NO_2.HCl)_2.PtCl_4$.

(b) The filtrate from the mercuric precipitate (F), freed from mercury by means of hydrogen sulphide, was precipitated with phospho-molybdic acid. The resinous precipitate after decomposition by means of lead acetate gave a hydrochloride crystallizing in colorless leaflets. The free base has a marked alkaline reaction, smells like ammonia, and exhibits a strong reducing action, precipitating metallic gold, for example, at once from a solution of gold chloride. The only salt that crystallizes well is the picrate which forms compact prisms melting at 195° and having the formula $C_8H_{11}NO.C_6H_2(NO_2)_3OH$. Brieger called the non-toxic free base mydine. Quite remarkable is the fact that apparently no very poisonous substance is formed during putrefaction of parts of human cadavers, particularly of internal organs. At all events Brieger obtained no such poison in following his method. Apparently access to a certain quantity of atmospheric oxygen is essential before putrefactive bacteria can form toxines of high toxicity.

II. Ptomaines from Four Months Old Horse Flesh

Examination of horse flesh that had undergone putrefaction during four months gave the following results:

(a) **Mercuric Chloride Precipitate.**—This gave considerable cadaverine and putrescine, a little mydatoxine, and a base having the formula $C_7H_{17}NO_2$. The latter together with mydatoxine was found in the mother-liquor from mercuric chloride double salts of cadaverine and putrescine. The last traces of putrescine were removed by decomposing mercury salts by means of hydrogen sulphide, dissolving the evaporated filtrates repeatedly in absolute alcohol, and allowing the alcoholic solution to stand for a long time. The alcoholic solution free from putrescine was again precipitated with alcoholic mercuric chloride solution and the precipitate repeatedly extracted with not too large a quantity of water. Upon standing difficultly soluble cadaverine mercuric chloride crystallizes from the hot filtered liquid, whereas easily soluble mercury compounds remain in the filtrate. Removal of mercury from the latter by means of hydrogen sulphide permits isolation of two ptomaines from the filtrate from mercuric sulphide. They may be separated by evaporating the filtrate to a syrup and adding gold chloride. The gold chloride double salt difficultly soluble in water crystallizes in leaflets. This compound when pure melts at 176° and has the formula $C_7H_{17}NO_2.HCl.AuCl_3$. Upon recrystallization it is sometimes obtained in needles but these may be reconverted into leaflets. The hydrochloride of the base, $C_7H_{17}NO_2.HCl$, crystallizes in fine needles that are insoluble in absolute alcohol. This base is toxic having a curare-like action upon frogs. Mydatoxine, $C_6H_{13}NO_2$, formed in human cadavers only in very small quantity, is obtained from the liquors filtered from the crystallized chlor-aurate after precipitation of gold

by means of hydrogen sulphide. The hydrochloride of this base forms a colorless, deliquescent syrup that yields no chlor-aurate with gold chloride. On the other hand, with platinic chloride it readily yields a chloro-platinate that dissolves easily in water and is obtained pure after repeated crystallization from absolute alcohol containing hydrochloric acid. Then it melts at 193° and corresponds to the formula $(C_6H_{13}NO_2.HCl)_2.PtCl_4$. Mydatoxine is toxic and resembles curare but is not very powerful in its action. Its toxic action is similar to that of the compound $C_7H_{17}NO_2$, associated with which it occurs in putrid horse flesh. The toxic phenomena exhibited by this ptomaine develop very slowly. Consequently experimental animals such as guinea pigs often do not die until 12 hours have elapsed. On the other hand, white mice are exceedingly sensitive to both poisons, reacting to both in the same manner.

(b) **Filtrate from Mercuric Chloride Precipitate.**—The precipitate produced by adding phospho-molybdic acid to the filtrate, freed from mercury by means of hydrogen sulphide, was decomposed by lead acetate, lead removed by hydrogen sulphide, and the filtrate evaporated with a little hydrochloric acid. Sodium picrate produced a resinous precipitate which was extracted hot with considerable water and brought to the form of felted needles. These dissolved in water with great difficulty and finally after repeated crystallization from boiling absolute alcohol yielded a picrate melting at 192° that was found upon analysis to be pure. This picrate having the formula $C_2H_7N_3.C_6H_2(NO_2)_3OH$ was dissolved in water, hydrochloric acid added, and picric acid completely extracted with ether. Evaporation of the aqueous solution with gold chloride and repeated crystallization of the product from water containing hydrochloric acid gave a chlor-aurate melting at 198° and having the composition $C_2H_7N_3.HCl.AuCl_3$. The hydrochloride obtained from this chlor-aurate by means of hydrogen sulphide, when treated with moist silver oxide, gave a highly toxic base that was colorless, deliquescent and strongly alkaline. It is identical with methyl-guanidine, $HN = C(NH.CH_3)(NH_2)$, the toxicity of which had already been established by Baumann and Herzens. For purposes of comparison Brieger prepared methyl-guanidine from creatine and found the synthetic product identical in all its properties with the ptomaine isolated from putrid horse flesh.

Separation of Cadaverine and Putrescine

(Baumann and v. Udránszky[1])

These two diamines, shaken in aqueous solution with benzoyl chloride and excess of 10 per cent. sodium hydroxide solution, are converted into dibenzoyl-compounds which crystallize well:

$$(CH_2)_4(NH_2)_2 + 2C_6H_5.CO.Cl + 2NaOH = (C_6H_5.CO.NH)_2(CH_2)_4 + 2NaCl + 2H_2O$$

Even very small quantities of cadaverine and putrescine may be detected by means of this reaction.

[1] E. Baumann and L. v. Udránszky: Benzoyl Chloride as a Reagent. Ber. d. Deutsch. chem. Ges. 21 (1888), 2744.

Procedure.—Use 5 cc. of benzoyl chloride and 40 cc. of sodium hydroxide solution (10 per cent. NaOH) for every 100 cc. of solution to be tested and shake vigorously until odor of benzoyl chloride has gone. The reaction of the mixture should be alkaline. A test should be made from time to time and, if the solution becomes acid, more sodium hydroxide solution should be added. After 24 hours collect any precipitate that has formed, wash thoroughly with cold water until free from alkali, dissolve in a little alcohol and pour the solution into water. The first indication is a milky turbidity. Upon long standing this changes to a crystalline precipitate consisting of dibenzoyl-compounds of the diamines present. Their separation is brought about by fractional crystallization. For this purpose add excess of ether to the alcoholic solution of the crystals. Dibenzoyl-putrescine crystallizes, whereas the filtrate upon evaporation gives dibenzoyl-cadaverine. If necessary, this procedure should be repeated. The dibenzoyl-compound of cadaverine, or pentamethylene-diamine, $(C_6H_5.CO.NH)_2(CH_2)_5$, melts at 130° and that of putrescine, or tetramethylene-diamine, $(C_6H_5.CO.NH)_2(CH_2)_4$, at 176–177°.

The free diamines may be obtained from their dibenzoyl-compounds by hydrolyzing with alcoholic potassium hydroxide solution.

Simulation of Alkaloids by Ptomaines

An Aconitine-like Ptomaine.—Mecke[1] isolated from a cadaver a ptomaine that showed a striking resemblance to aconitine. Ether extracted it from alkaline aqueous solution. A very dilute solution in weak hydrochloric acid gave the general alkaloidal reactions, that is, a white precipitate with phospho-tungstic acid, a yellow precipitate with phospho-molybdic acid, a brown precipitate with iodo-potassium iodide. It remained clear with tannic acid and gave a faint turbidity with potassium mercuric iodide.

Special Reactions.—This substance gave a yellowish color with concentrated sulphuric acid. After long standing the color changed to rose-violet and became darker upon warming. This ptomaine remained colorless with dilute sulphuric acid but developed a violet color upon evaporation. The same result was given when 25 per cent. phosphoric acid was used. The solution with Froehde's reagent had a greenish color that changed to yellow-brown upon warming. Chemical identification of aconitine in cadaveric material is quite uncertain by the methods thus far used, unless the alkaloid is present in considerable quantity. Moreover the examination must be confined to material that is entirely fresh. The Herbst reaction with phosphoric acid not only is slightly characteristic of aconitine but does not give the same result with different commercial varieties

[1] Mecke: A Cadaveric Alkaloid Similar to Aconitine. Zeitschr. f. öffent. Chem. 5 (1899), 204.

of aconitine. Furthermore when ptomaines are present, its use is entirely excluded.

Cadaveric Coniine.—In the past this substance has been described many times. In most instances, however, examiners were probably dealing with cadaverine and putrescine. For this reason a physiological test upon a frog should always be made in case of doubt. Coniine injected into a frog to the extent of at least 15 mg. has a curare-like action. The muscles are relaxed and quiver. The gait is unsteady. Death ensues from asphyxiation due to paralysis of the respiratory muscles.

A Codeine-like Ptomaine.—Gadamer (Chemical Toxicology) mentions a ptomaine resembling codeine in its properties. Ether extracted it from alkaline solution. Evaporation of the hydrochloric acid solution with sulphuric acid gave, it is true, a red residue but did not give Pellagri's reaction.

A Colchicine-like Ptomaine.—Baumert[1] isolated such a substance from a cadaver 22 months after death. This ptomaine so closely resembled colchicine in solubility and reactions that it could be positively differentiated from that alkaloid only by failure to give the ferric chloride test and by the fact that it could be precipitated by picric acid.

A Strychnine-like Ptomaine.—During a forensic-chemical investigation Mecke and Wimmer[2] found such a ptomaine in a cadaver that had already reached a high state of putrefaction. This ptomaine appeared in white, wart-like crystals that as far as chemical reactions were concerned might very easily have been mistaken for strychnine. Ether extracted it from akaline aqueous solution. Concentrated sulphuric acid produced a yellow solution changing to cherry-red and rose-red (such a strychnine solution is colorless). The yellow, crystalline precipitate given by potassium dichromate dissolved in concentrated sulphuric acid with blue color. The yellow, crystalline precipitate given by potassium ferricyanide dissolved in concentrated sulphuric acid with violet color. Finally the yellow, crystalline precipitate given by picric acid produced a violet color with concentrated sulphuric acid and potassium dichromate. This ptomaine differed from strychnine in dissolving in Froehde's

[1] G. Baumert: A Putrefactive Product Similar to Colchicin. Arch. d. Pharm. 225 (1887), 911.

[2] Mecke and Wimmer: A Cadaveric Alkaloid Similar to Strychnine. Pharm. Ztg. 43 (1898), 300.

reagent with a dirty violet color that changed later to olive and green (strychnine gives a colorless solution). The solution in Erdmann's reagent was yellow (strychnine is colorless). Finally this ptomaine had only a slightly bitter taste and was without tetanizing action upon the frog. G. Baumert[1] has expressed the opinion that up to the present time no ptomaine has been found that entirely resembles a plant-base, that is, in both physiological and chemical properties.

A Veratrine-like Ptomaine.—Stüber[2] has described a ptomaine having properties like those of veratrine. An epidemic on shipboard among the rats nearly caused their extermination but a bacteriological examination failed to show plague bacteria or any other bacterial cause of death. Fresh cadavers of several rats were extracted in the usual manner with tartaric acid and alcohol according to the Stas-Otto process. Ether extracted from the alkaline aqueous solution a basic substance. After purification this substance was yellow and amorphous and exhibited the following properties. Warmed with concentrated hydrochloric acid, it developed the splendid cherry-red color given by veratrine. Its solution in concentrated sulphuric acid was first yellow, then orange and finally violet. It failed, however, to give Weppen's test characteristic of veratrine, that is, a blue color with sugar and concentrated sulphuric acid. Injected subcutaneously into a frog, this ptomaine failed to produce muscular twitching characteristic of veratrine. Completely rotten maize that may have been eaten by the rats on shipboard yielded no basic substance resembling veratrine. Brouardel and Boutmy as well as Béchamp[3] before that had observed ptomaines that resembled veratrine.

[1] G. Baumert: Private Communication.

[2] Stüber: A Ptomaine Similar to Veratrine. Zeitschr. f. Unters. d. Nahrgs.- u. Genussm. 6 (1903), 1197.

[3] J. Béchamp: Occurrence of Ptomaine-like Products as a Result of Peptic and Pancreatic Digestion of Several Protein Substances. Compt. rend. 94 (1882), 973.

CHAPTER V

APPARATUS, SPECIAL METHODS OF EXAMINATION AND SPECIAL EXAMINATIONS

Certain Apparatus

Extractors

Extraction of an alkaloid or other substances from aqueous solution by a solvent immiscible with water, such as ether, chloroform, benzene, or petroleum ether, is usually effected by thoroughly shaking together and separating the two liquids in a separatory funnel. The less soluble in water the substance to be extracted is and the more soluble in the given solvent (ether or chloroform), the more easily it will pass from water to solvent. Three or four thorough extractions, each time with a fresh portion of solvent, usually suffice to transfer the given substance to the organic solvent almost quantitatively, at least for practical purposes. Alkaloids exhibit such solubility relations in water and ether, benzene or chloroform. Their coefficient of solubility in water is considerably less than in these organic solvents. A substance soluble in two immiscible solvents will distribute itself between the two, when thoroughly shaken together, so that both are equally saturated. If the coefficient of solubility of a substance in chloroform is 20 times greater than in water at the same temperature, then with equal volumes of water and chloroform, after thorough shaking, 20 times more of the substance will be dissolved in chloroform than in water. Water containing 0.2 gram of a given substance, when shaken with the same volume of chloroform until saturated, will transfer 0.1905 gram to the chloroform and retain only $0.2 - 0.1905 = 0.0095$ gram.[1] This example will serve to show that practically the substance will be

[1] **Calculation.**—If x is the quantity of substance dissolved in chloroform and y the quantity dissolved in water, then: I. $x + y = 0.2$; II. $x:y = 20:1$. From this it follows that $y = \frac{x}{20}$ and by substitution of this value of y in I: $x + \frac{x}{20} = 0.2$, $\frac{21}{20}x = 0.2$ and $x = 0.1905$; $y = 0.2 - 0.1905 = 0.0095$.

transferred after 2–3 extractions with equal volumes of chloroform from water to chloroform. When conditions are reversed, that is, if a substance dissolves with greater ease in water than in an organic solvent, relations will be different. Then an infinite number of extractions with a given solvent will be required for complete transfer of substance from water to organic solvent. In no other way will the error become infinitely small. In that case it is advisable to use an extractor permitting a great many extractions in one operation. This apparatus is very useful in many examinations of urine, foods and beverages. The extractors best known and deserving special recommendation are mentioned below.

Gadamer's universal extractor[1] (Fig. 31) may be used with equal advantage to extract solutions either with solvents lighter than water, such as ether, benzene, petroleum ether, etc., or with solvents heavier than water, such as chloroform. The apparatus shown in the drawing with cock *f* closed may be used for extractions with ether and other solvents lighter than water. Tube *a* cut at a bevel is connected with a boiling-flask. Introduce 30–40 cc. of the liquid to be extracted into the system *de* through the funnel *c*. Add a sufficient quantity of ether and close the wider tube *b* with a Soxhlet ball-condenser. The author prefers a glass ball-condenser like that connected with the Blas-Bosmann extractor (Fig. 32), because it completely condenses ether vapor. Ether boiling in the flask passes through tube *a* (Fig. 31), condenses in the cooler and flows into the funnel-tube *c*. As soon as the hydrostatic pressure of ether overcomes that of the liquid undergoing extraction, ether trickles through the latter, collects on top and flows through tube *a* back into the boiling-flask. The dead space above cock *f* is eliminated by filling with pure mercury. Thus a substance may be completely extracted in a relatively short time. Even substances, such as formic, acetic and lactic acids, extracted only with greatest difficulty and very incompletely, may be removed almost quantitatively from aqueous solution by means of Gadamer's extractor.

The apparatus is inverted when using a solvent heavier than water, such as chloroform. When cock *f* is opened and the end of tube *a* cut on a bevel is closed by a stopper, the apparatus is ready for extraction. Connect the widened tube *b* with the boiling-flask and the widened tube *g* with the Soxhlet ball-condenser. Pour chloroform, or any other solvent heavier than water, through *g* until it flows into the boiling-flask and then introduce the liquid to be extracted into the apparatus preferably by means of a long-necked, fine dropping funnel. Since tube *a* is closed and filled with air, vapor from boiling chloroform must pass through the funnel *c* to *g* into the cooler where it is condensed. Then liquid chloroform passes through the wide bore of the cock, drops upon the liquid, and slowly makes its way down. As with ether, rapid extraction is brought about by frequent and intimate contact of chloroform with the aqueous solution.

[1] The universal extractor of J. Gadamer is furnished by the firm of Paul Altmann, Berlin.

Gadamer's universal extractor is quite practical but has the disadvantage that it cannot be used to extract large volumes of liquid. The Blas-Bosmann extractor (Fig. 32) for ether extractions and the Pregl extractor (Fig. 33) for chloroform extractions are adapted for such purposes. Both may be made of any size desired.

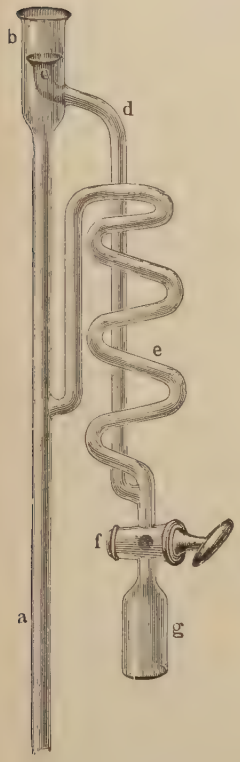


FIG. 31.—Gadamer's universal extractor.

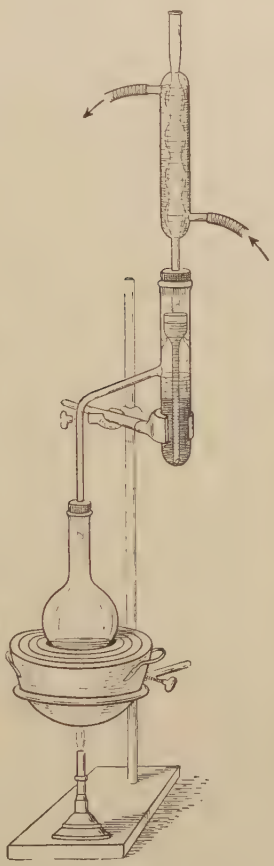


FIG. 32.—Blas-Bosmann ether extractor.

FIG. 33.—Pregl[1] chloroform extractor.

Liquid undergoing extraction in the ether extractor is covered with a layer of ether. Then the ether in the flask is heated to boiling upon a water-bath. Vapor condensed in the cooler drops through the funnel to the bottom of the extraction-tube. From there it rises more or less finely divided through the aqueous solution to the top.

[1] Taken from Pharmaceutische Chemie II by E. Schmidt.

In using the chloroform extractor (Fig. 33) first introduce chloroform (a) and then add as an upper layer the liquid to be extracted (b). Heat the chloroform in the flask to boiling upon a water-bath. The vapor may be condensed by means of a Soxhlet ball-condenser, or any other similar apparatus may be used. The condensed chloroform falls in finely divided condition through the liquid undergoing extraction.

Autenrieth-Koenigsberger Wedge-Colorimeter[1]

For photo-colorimetric measurements physicists have devised very fine, accurate instruments, such as Wild's, Lummer and Brodhun's, and Koenig's spectro-photometer. These instruments are quite expensive, and involve manipulation and calculations that are rather troublesome. In making very careful measurements these finer spectro-photometers have always been of great service to physicists. The wedge-colorimeter[2] to be described is a handy instrument of general application, giving results that are sufficiently accurate, at least for practical purposes. An essential part of this colorimeter is the hollow glass wedge (K) (see Figs. 34 and 35). This is always filled with the solution of the color-standard required as a comparison solution in carrying out a colorimetric determination. This glass wedge is attached to a wooden frame together with a milk-glass plate (Mg) and a scale (S). By means of a rack and pinion this frame may be moved up and down. Close to the glass wedge is a small, immovable glass trough (T.) In this is put the solution obtained from the given material to be examined colorimetrically. The entire optical apparatus is enclosed in a wooden case, the front of which is provided with a blackened movable shutter. This has a slit 1 cm. high and 2 cm. wide for an observation window. Just behind the latter and directly in front of the glass-wedge and glass-trough is placed a Helmholtz double-plate (D.P.) to prevent formation of a dark space or even of a dark line between the two color solutions in the glass-trough and glass-wedge that are being compared colorimetrically. The passage of the light rays is shown in Fig. 36. The result of the introduction of the double plate is that the two color bands being compared overlap. Thereby the dark

[1] W. Autenrieth and J. Koenigsberger: A New Colorimeter and Its Use in the Determination of Blood Pigment, Creatinine and Iron. Münchener med. Wochenschr. 57 (1910), 998.

[2] The wedge-colorimeter and carefully calibrated comparison-wedges with calibration-curves are supplied by the firm of F. Hellige and Co., Freiburg i. B., Albertstrasse.

line of separation that otherwise would be unavoidable is eliminated. When such a dark interval appears between the two color solutions, as it does in certain simpler colorimeters, the accuracy of the colorimetric comparison, as photometric measurements have shown, is diminished by half and less. Attached to the wooden frame, is also a pointer (Z) past which the scale on the frame may be moved up and down by means of the rack and pinion. The color solution to be compared colorimetrically, which must always be absolutely clear, is poured into the glass trough. The colorimeter is then held against the sky, or a white wall, but not where the sunlight is too glaring, so that the field of vision is distinct. The glass-wedge is moved up and down until the two color solutions being compared colorimetrically have exactly the same depth of color. To establish identity of color, four to six readings should be made and the arithmetical mean of these taken as the colorimetric determination. Frequently the first and even the second reading will be incorrect, because the eye must become accustomed by trial to the particular shade of the color solution. For that reason rejection of the first two readings and acceptance of the following three or four readings is advisable.

As compared with the spectro-photometers mentioned above and the simpler colorimeters, the wedge-colorimeter has the following advantages:

A determination may be made very rapidly. This is so at least when the glass-wedge required for a definite colorimetric method and filled with the comparison solution has already been calibrated. Calibration of a comparison wedge and construction of the calibration curve have to be done only once. If there is reason to suspect that the strength of the color solution in the comparison wedge has changed, a few points on the curve may be redetermined and, if necessary, a new curve constructed. In most instances calibration of the comparison wedge, including construction of the curve, should not take longer than the accurate adjustment of a normal solution. The great accuracy possible in the readings should be emphasized, for usually the point of equal color strength may be established to within 1–2 divisions on the scale.

The fact that the two containers required for colorimetric comparison, that is, the glass trough and the glass wedge have parallel walls is an additional advantage of the wedge-colorimeter. For this reason intensity of color is the same at all points of a given cross-section, because the depth of the layer is the same. Another advantage of this over other forms of apparatus, that should not be overlooked, is the possibility of comparing colorimetrically color solutions of any concentration whatever, provided they are within the measuring range of a calibrated comparison wedge. Consequently there is no need of diluting a color solution until its strength is the same as that of a comparison solution which frequently is an operation resulting in too great dilution.

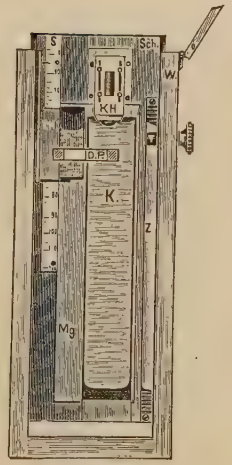


FIG. 34.

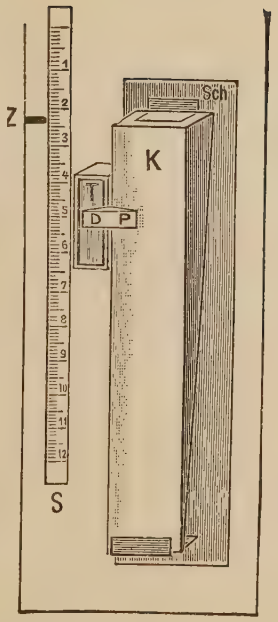


FIG. 35.

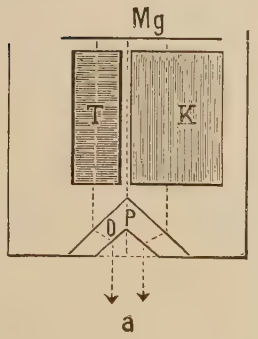


FIG. 36.

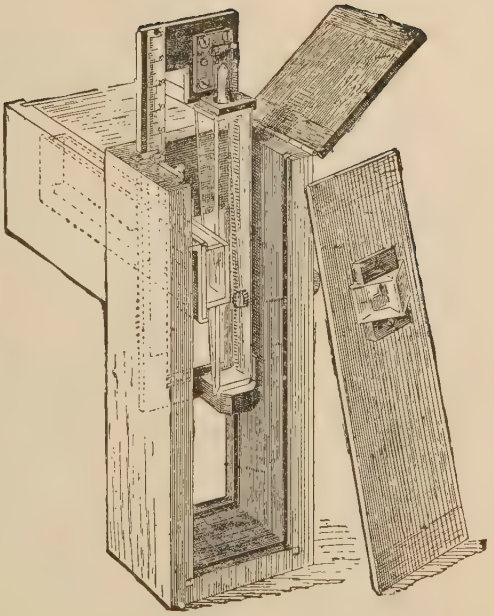


FIG. 37.

FIGS. 34–37.—Autenrieth-Koenigsberger Colorimeter.

FIG. 34.—Colorimeter as a whole.

FIG. 35.—Sch. = slide (rear view) with glass wedge K and scale S; T = glass trough, Z = pointer, DP = Helmholtz double plate.

FIG. 36.—Passage of light rays. Mg = milk glass plate, K = glass wedge with standard solution, T = glass trough with liquid to be examined colorimetrically, DP = Helmholtz double plate, a = the eye.

FIG. 37.—Colorimeter with long glass trough and light screen.

The final operation is the preparation of the standard color solutions used to fill the hollow glass wedges that serve as "comparison wedges." These are artificial color solutions that are permanent. Within the measuring range of the given wedge they have the same, or nearly the same, shade of color as that of the solutions to be examined colorimetrically. Color solutions that keep may be easily prepared by mixing solutions of colored inorganic salts, such as chrome alum, iron alum, copper sulphate, copper tetramine sulphate, potassium dichromate and chromate, and colbaltous nitrate. In many cases picric acid solution, which remains unchanged longer than had previously been supposed, may be used to prepare such color solutions.

Calibration of the Comparison Wedge.—Obviously each colorimetric method carried out by means of the wedge-colorimeter calls for a glass wedge filled with a permanent color solution. This wedge must be calibrated against a chemically pure preparation of the given substance in the determination of which in a given material it is to be used. The manner of doing this has been accurately described under each substance that has been determined colorimetrically. The colorimetric methods taken up in this book are: determination of arsenic, bromine in bromides, iodine in iodides and in the thyroid gland, adrenaline in the suprarenal gland and in suprarenine preparations, hydrocyanic and formaldehyde in distillates, morphine in opium preparations, mercury and salvarsan in urine, and K-strophanthine in strophanthus tincture. In a quantitative colorimetric determination of traces of a compound, for example, ammonia or hydrogen sulphide in river or drainage water, a glass trough five times longer should be used to hold the given color solution. One end of the trough with a milk-glass plate as a background should be enclosed in a wooden case as a light-screen. A colorimeter of this description is shown in Fig. 37. The long glass trough with light-screen may readily be put into this form of the colorimeter. The only change necessary is first to remove the milk-glass plate on the wooden frame and substitute another holder for the long glass trough.

Eder's[1] Micro-sublimation of Alkaloids in Vacuo

Careful experiments by Eder have shown that most of the alkaloids, especially those of forensic importance, may be sublimed *in vacuo* almost without decomposition. Such micro-sublimates frequently have a very characteristic appearance and may be used for recognizing certain alkaloids microscopically.

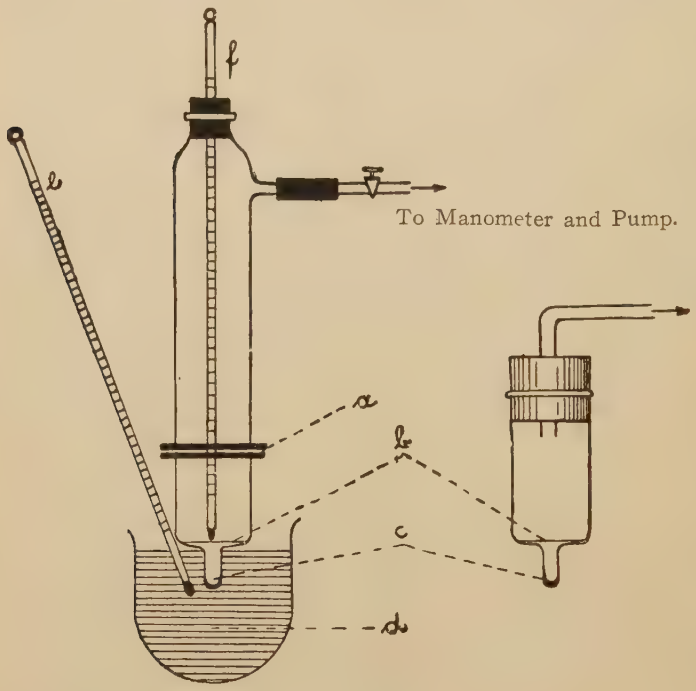
Apparatus.—Since very small quantities of material usually have to be sublimed, sublimates should be deposited upon a small surface that may be easily examined under the microscope. Comparable results in sublimation are possible only when attention is paid to temperature. Consequently the apparatus should be so constructed that the temperature may be measured approximately both where the substance is vaporized and where the sublimate is

[1] R. Eder: Micro-sublimation in Vacuo. Zurich 1912.

deposited. The micro-sublimation apparatus[1] constructed by Eder, as shown in the drawing, has been found practical and convenient. Such an apparatus, constructed of the most resistant Jena glass of highest quality, has given excellent service.

Eder's Micro-sublimation Apparatus

This apparatus (Fig. 38) consists of two parts, both of which are made of glass tubing 2.5 cm. in diameter. The shorter and lower



[Fig. 38.

part (4.5 cm. long) is reduced at the bottom to a little cup 1 cm. deep and 0.5 cm. in diameter. The substance (c) to be sublimed is placed in this. A round cover-glass (b) 18 mm. in diameter is placed over the cup to collect the sublimate. This little plate is 13 mm. from the bottom of the cup. The open end of the lower part of the

[1] This apparatus is supplied by the glass-blowing establishment of A. Wittmann, Zurich IV, Sonneggstrasse 2.

apparatus has a thick flat edge well-ground and fitting tightly to the upper part. The latter is 12 cm. long and closed at the top by a short rubber stopper through which passes a thermometer (f). The bulb of the thermometer comes as close as possible to the round cover-glass in the lower part of the apparatus. The upper part, held in a perpendicular position by a clamp, is provided with a side-tube connected by vacuum tubing with a manometer. It is also connected with a water-pump. Between the pump and manometer are a drainage glass-cock and a thick-walled suction-flask to prevent water from being sucked back from the pump into the manometer. If the ground surfaces fit well and are slightly greased, a good pump will give a vacuum of 8–12 mm. The ground union may be dispensed with, if sublimation is carried on in the smaller apparatus also shown in the drawing. If the temperature of the little plate is not taken, the inner thermometer may be omitted. When the temperature of the cup is much above the melting-point of the substance being sublimed, obviously the temperature of the little plate will be less than that of the cup. The apparatus is so heated that only the lowest part, that is, the cup containing the substance, is immersed in concentrated sulphuric acid. The latter is slowly warmed in a beaker by means of a small flame. It is quite important that the substance to be sublimed be first finely powdered and carefully dried in desiccator. A vacuum desiccator is to be preferred. This will exclude appearance of water vapor to interfere with the formation of the sublimate. The substance in the cup not exceeding 0.5 mg. should be stirred with a dry glass rod to bring it uniformly into close contact with the walls of the cup. Unless the substance is close to the glass walls, the heat will not act uniformly upon every particle and the sublimate in all cases will not form at about the same temperature. If the substance is dissolved in alcohol, ether, or chloroform, the solution may be dropped into the cup by means of a pipette, evaporated upon a water-bath, and the residue very carefully dried in a vacuum desiccator before being sublimed. When the substance is in the cup, place over it with forceps a clean, round cover-glass 18 mm. in diameter that is absolutely dry. Eder cleans the cover-glass in chromic acid mixture, rinses it in water, and dries with a very fine cloth.

When the lower part of the apparatus is equipped with substance and cover-glass, start the pump and connect the lower with the upper part so that a vacuum is produced. The pump should be

kept running throughout the operation! As soon as the manometer registers 8–12 mm., immerse the cup in sulphuric acid so that the acid comes to a point about midway between the bottom of the cup and the cover-glass. At the same time place a thermometer (e) in the bath. The rise of the vapor depends absolutely upon immersion of the cup in the bath always at the same depth. If it is too deep, a sublimate sometimes forms on the walls of the apparatus above the little plate. The sulphuric acid bath should be heated very slowly by means of a Bunsen flame about 2 mm. high. About 8 minutes should be required to raise the temperature of the acid from 25° to 100°. During heating the little plate should be carefully watched with the naked eye and through a magnifying glass. When the manner of heating is the same, a given substance will always begin to appear as a sublimate within a definite range of temperature. As soon as the first appearance of a sublimate is established, the bath should be heated 30–50° higher and the experiment then stopped. The sublimation apparatus should first be removed from the acid, the glass stop-cock closed between manometer and pump, and the latter stopped. Grasp the lower part of the sublimation apparatus with the left hand and with the right hand slowly and carefully open the cock of the manometer to let air into the apparatus. Then take off the lower part and remove the cover-glass with forceps. Clean the cup with chromic acid mixture. The sublimate may be identified either under the microscope or by microchemical reactions.

On the basis of behavior during sublimation, first appearance of a sublimate, and changes that may take place, Eder has divided the alkaloids he examined into three main groups:

A. **Those That Sublime without Melting.**—Sublimation of alkaloids of this group appears at least to follow the following course:

Crystalline → Vapor Phase → Crystalline

Examples of such behavior are caffeine, theobromine, cinchonine, solanine and cantharidin.

Included in this group also, are substances that do not form a crystalline sublimate at first. Under the microscope fine, amorphous droplets are seen. These become crystalline. Sublimation in this case follows the course:

Crystalline → Vapor Phase → Amorphous Droplets → Crystalline

This group includes most of the alkaloids: for example, morphine, strychnine, codeine, quinine, narcotine, thebaine, yohimbine.

Finally this group contains substances that sublime in fine, amorphous droplets which do not uniformly become crystalline. Sublimation follows the course.

Crystalline → Vapor Phase → Droplets → Eventually Crystalline

Among the more important alkaloids belonging here are: atropine, brucine, cocaine, homatropine, hydrastine, papaverine, physostigmine.

B. **Those That First Melt and Then Sublime.**—In this case at temperatures above their melting-points these substances sublime in fine, amorphous droplets that do not become crystalline according to the scheme:

Solid → Liquid → Vapor → Amorphous Droplets

Examples of such behavior are: colchicine, emetine, narceine, pilocarpine, veratrine.

C. **Those That Form No Sublimate.**—Many salts of alkaloids belong in this group, for example, nicotine hydrochloride.

For information with regard to the microscopic examination of micro-sublimates, Eder's original paper containing 20 photo-micrographs should be consulted. If doubt exists as to whether the sublimate is a certain alkaloid or not, a micro-sublimate of pure, known alkaloid should be prepared and carefully compared with that given by the material in question. Various text-books[1] furnish details regarding the methods used in carrying out micro-chemical reactions. For example, Behrens gives the following two micro-chemical reactions for the recognition of caffeine and these are given very well by micro-sublimates:

1. **Mercuric Chloride.**—Dissolve the sublimate with a small drop of about 0.5 per cent. hydrochloric acid. Then add by means of a platinum needle a trace of pure mercuric chloride at the margin of the drop. Almost instantly long, colorless, highly refracting needles of caffeine chloro-mercurate shoot out, usually in the form of radiating spheroids.

2. **Silver Nitrate.**—Dissolve the micro-sublimate in a small drop of very dilute nitric acid. Then add by means of a platinum needle a trace of powdered silver nitrate at the margin of the droplet. Gray, woolly or moss-like clusters of thin crystalline threads, caracteristic of caffeine-silver nitrate, quickly appear first at the margin and soon extend within the droplet.

The micro-sublimate of strychnine gives the bichromate-sulphuric acid reaction and Mandelin's test very beautifully. Also the morphine sublimate gives color reactions very well with the reagents of Froehde and Marquis, as well as Husemann's reaction. The same is true of the codeine sublimate. The quinine sublimate gives the herapathite, thalleioquin and erythro-quinine reactions very beautifully. The first is given especially well, if the modified method (Grutterink[2]) devised by Behrens is adopted: Mix equal parts of water, alcohol, and dilute acetic acid, adding to this mixture some sulphuric acid and sufficient potassium iodide to produce a faint yellow color. A drop of this solution upon a sublimate causes the immediate appearance of small plates, irregular diamond-shaped herapathite crystals, of a pale rose, crimson-red to violet color. These appear greenish in reflected light and very strongly dichroic in polarized light. Quinidine, cinchonine and cinchonidine do not give the herapathite test. The atropine sublimate gives the reaction depending upon formation of the hydri-

[1] E. M. Chamot: Elementary Chemical Microscopy. John Wiley, New York, 1915.

[2] A. Grutterink: Contribution to the Microchemical Analysis of Certain Alkaloids and Drugs. Inaugural Dissertation, Berne, 1911.

odide of atropine tetriodide and especially well Eder's bromine-potassium bromide test (see Atropine).

According to Eder, all alkaloids that can be sublimed form a sublimate consisting of fine amorphous droplets as the first condensation product of the vapor. These are an unstable, under-cooled form of the given alkaloid that changes into the more stable, crystalline form. The speed at which individual alkaloids change in this respect is very different. Experiment has shown, however, that the farther an alkaloid sublimes below its melting-point, that is, the greater its vapor-pressure, the less stable are the amorphous droplets and the more certain and rapid is crystal formation in the amorphous sublimates.

In many instances micro-sublimation has unquestionably been of great service in legal-chemical practice, since it is not only a method of isolation but an excellent method of purification. The value of the purity of sublimates obtained as a result of micro-chemical procedure is especially shown by the fact that micro-chemical reactions and color reactions may be made with them much better than with the ordinary alkaloidal residues in watch-glasses. Eder, however, has correctly observed "that alkaloidal residues obtained in toxicological analysis, when impure on account of greasy, resinous or fatty substances, should first be purified as carefully as possible by the usual solution methods and finally subjected to micro-sublimation as the last step in purification."

A. INORGANIC CHEMICAL EXAMINATIONS

Electroscopic Detection of Minute Quantities of White Phosphorus, Especially in Presence of Phosphorus Sesquisulphide

(Schenk and Scharff[1])

This method[2] of detecting phosphorus is based upon the fact that white phosphorus, even in very small quantities, ionizes air so that it becomes a conductor of electricity, whereas phosphorus sesquisulphide, P_4S_3, even when luminous is unable to render air a conductor. Consequently "phosphorus air" will discharge a charged electroscope, whereas phosphorus sesquisulphide has no action whatever upon the electroscope at temperatures up to 50° and no appreciable action up to 75°. This method is also applicable to the detection of white phosphorus in any part of the cadaver, as, for example, in stomach and intestinal contents, in remnants of food, or in vomitus. The apparatus in the drawing devised by Schenk and Scharff is very useful as a hygienic control in factories engaged in using white

[1] R. Schenk and E. Scharff: A Method for the Detection of Minute Quantities of White Phosphorus. Ber. d. Deutsch. chem. Ges. 39 (1906), 1522.

[2] At the end of this chapter, page 574, are given "Official Directions for the Chemical Examination of Matches for Their White or Yellow Phosphorus Content."

phosphorus. By means of this apparatus it is easy to determine whether air in rooms meets hygienic requirements or not, that is, whether it contains a sufficient quantity of the oxidation products of phosphorus to give rise to necrosis. But distillates obtained by the Mitscherlich, or Scherer-Fresenius-Neubauer method, are not suitable for this purpose, for during distillation phosphorus sesquisulphide gives off small quantities of phosphorus that pass over into the distillate.

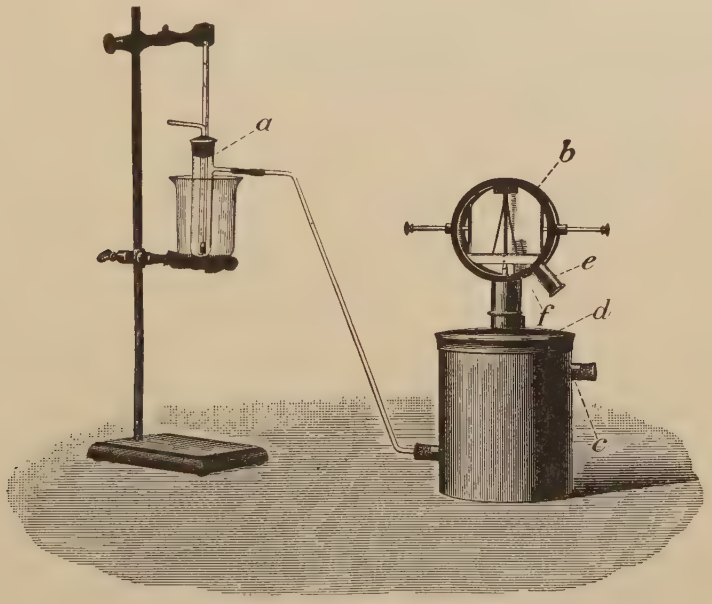


FIG. 39.—Schenk and Scharff apparatus for detecting white phosphorus.

Apparatus.[1]—The complete apparatus (Fig. 39) is made up of the glass parts (a), the electroscope (b), and the condenser (c). The vessel for producing phosphorus-air comprises a test-tube provided with a side-arm and ground-glass stopper. A tube for introducing air passes through the latter and extends almost to the bottom of the test-tube. Attached to the side-arm by means of a rubber connector is a tube conducting the phosphorus-air to the condenser. The cylindrical condenser (c) is provided with openings on the side that

[1] The complete apparatus is supplied by the mechanician of the Physiological Institute of the University of Marburg.

permit gases to be passed through. The detachable cover (d) carries the electroscope. The insulated diverging leaves are suspended within the outer cylinder. The interior of the electroscope-box naturally should be carefully protected from phosphorus oxidation products. Two well-insulated small plates through which the carrier of the diverging leaves passes by means of a tight connection make this possible. They may be unscrewed and cleaned if necessary. On one side of the electroscope-box is an attachment (e) into which a piece of metallic sodium may be introduced to act as a drying-agent. Finally, passing through the cover of the cylinder is a movable, metallic charging-needle (f) insulated from the cover and provided with an insulated handle. Before a test is made, by means of the latter the diverging leaves are charged as highly as possible by a Zamboni-pile or an ebonite rod rubbed with silk. In charging the instrument the charging-needle is turned opposite to the hands of a clock and moved up to the diverging leaves. The metallic part is then touched with the Zamboni-pile or the excited ebonite rod. When the apparatus is charged, the charging-needle is at once removed by turning with the hands of a clock. The diverged aluminum leaves are set at the proper division on the scale by moving toward them the two metallic plates of the electroscope. A blank test should always be made by partially filling the test-tube (a) with water and warming in a water-bath (beaker) to 35–55°. Then a current of air is passed through and the fall taking place within 5 minutes is read off on the scale. The cylindrical condenser now wet as a result of the blank test is well dried. Then the material is introduced into the test-tube with a little zinc oxide and the same procedure as in the blank test repeated. If the fall of the diverged leaves is considerably greater within 5 minutes than in the blank test, the presence of elementary phosphorus may be inferred.

To detect the presence of white phosphorus in phosphorus sesquisulphide by the method of Schenk and Scharff, triturate about 0.2 gram of the solid mixture with zinc oxide and a little water. Introduce this triturate into test-tube (a) and apply the test described above. It has been found that 0.2 gram of this mixture, containing only 0.4 mg. of white phosphorus, is capable of exerting a powerful discharging action upon the electroscope. The degree of sensitiveness of this conductivity method is considerably higher in case of pure white phosphorus, for 0.004 mg. of pure white phosphorus still have an effect upon the electroscope.

Notes.—According to Schmidt,[1] solutions of phosphorus in oil are also capable of rendering air a conductor of electricity. The conductivity is proportional to the quantity of phosphorus present. The optimum temperature at which this takes place is 60°. Ionization of air decreases with oxidation of phosphorus, for in the case of oils a protective film of oil-insoluble oxide forms. Inactive gases, such as hydrogen, carbon dioxide and nitrous oxide, may become saturated with phosphorus vapor and then exert a very strong ionizing action upon air as soon as it enters into oxidation. Pure oxygen will also saturate itself with phosphorus vapor and oxidize the latter as soon as its dilution reaches the point at which strong ionizing action takes place.

Detection of Free White Phosphorus in Phosphorus Sesquisulphide

(Schroeder[2])

According to Schloesing,[3] phosphorus sesquisulphide containing only very little white phosphorus exhibits even at ordinary temperature when shaken in the dark more or less pronounced luminosity. An additional means of detecting white phosphorus is its solubility in petroleum ether. A liter of this solvent will dissolve 8 grams of phosphorus but only very little pure phosphorus sesquisulphide.

To distinguish white phosphorus from phosphorus sesquisulphide, Schroeder recommends dissolving the material in benzene. Benzene solutions of phosphorus sulphides spontaneously evaporated upon filter paper at ordinary temperature produce no luminosity, not even when the paper strip is rubbed with the fingers. But if the benzene solution is evaporated with heat, phosphorus sesquisulphide, P_4S_3, will give rise to luminosity upon the paper strip. Evaporation of sufficiently concentrated benzene solutions of pure phosphorus gives rise to luminosity even at ordinary temperature. The delicacy of the reaction is increased by rubbing the paper strip with the fingers. If the test is carried out in a particular way, that is, if the benzene solution is allowed to evaporate at 50°, pure phosphorus sulphide compounds usually will not render the surface of the paper luminous. On the contrary, preparations containing free white phosphorus not only render the paper strips luminous but cause the

[1] H. Schmidt: Information with Regard to Phosphorated Oils and their Combination in the Organism from the Electroscopic Detection of Phosphorus. Biochemische Zeitschr. 34 (1911), 280.

[2] F. Schroeder: Detection of White Phosphorus in Matches. Arbeiten a. d. Kaiserl. Gesundheitsamt 44 (1913), 1.

[3] Th. Schloesing Fils: Detection and Estimation of free White Phosphorus in Phosphorus Sesquisulphide. Compt. rend. de l'Acad. des sciences 155 (1913), 1461.

appearance of luminous mist. The latter, however, is not very distinct when the quantity of phosphorus is small. This reaction takes place only when the temperature is kept exactly at 50°.

Notes.—Under the conditions established by Schroeder, white phosphorus dissolved in benzene may still be detected when 1 cc. of solvent contains 0.01 mg. The delicacy of the luminescence test is lower when sulphur compounds of phosphorus are present than it is when pure phosphorus is dissolved in benzene. In order that the luminescence test may be given in presence of phosphorus sesquisulphide, the benzene solution must contain as much as four times the quantity of white phosphorus.

Detection of Traces of White Phosphorus in Presence of Large Amounts of Phosphorus Sesquisulphide

(Aronstein[1])

Hydrogen washed by means of potassium permanganate solution, or carbon dioxide washed by water, is dried by concentrated sulphuric acid and then mixed with dry air in a T-tube. The mixed gases are then passed over the phosphorus sesquisulphide, P_4S_3, to be tested for white phosphorus. If white phosphorus is present, luminescence appears when the current of gas contains only a little air. When more air is admitted, it disappears but will reappear if the supply of air is diminished. Pure phosphorus sesquisulphide will not behave in this manner unless the temperature is over 80°. This test is given by 0.2 per cent. of white phosphorus, provided the absolute amount is not less than 0.04 mg. If the material examined contains such impurities as oil of turpentine, alcohol, and other volatile compounds, the test will not appear until the current of gas has been passed over the material for several minutes. Material that has been kept 8 days or more without special precautions has also given the phosphorescence test.

Qualitative Detection of Phosphorus in Phosphorated Oils

1. Straub's[2] Test.—If a phosphorated oil is placed on the surface of copper sulphate solution, phosphorus will gradually pass from the oil to the aqueous solution and first form black copper phosphide. The latter acting as a carrier of oxygen oxidizes phosphorus still

[1] L. Aronstein: A Method of Detecting Small Quantities of White Phosphorus in Presence of Large Quantities of Phosphorus Sesquisulphide. Chem. Weekblad 3 (1906), 283.

[2] W. Straub: A Simple Method of Detecting Phosphorus in Phosphorated Oils for Clinical Purposes. München. med. Wochenschr. 50 (1903), 1145.

in the oil to phosphoric acid which dissolves in water. Detection of phosphorus in phosphorated oils by Straub's method is based upon this behavior. Shake 10 cc. of phosphorated oil in a test-tube with 5 cc. of copper sulphate solution (5 per cent. $CuSO_4.5H_2O$). According to the amount of phosphorus, a black or light brown coloration will appear in the emulsion at once, or in a few minutes, and at most after 2 hours.

A practical, therapeutic application of this reaction may be made in acute phosphorus poisoning. Administration of copper sulphate solution may prevent absorption of free phosphorus still in the stomach and intestinal tract.

2. **Mitscherlich Test.**—This test is also applicable to phosphorated oils, even though the oil may contain only 0.2 mg. of phosphorus in 100 cc. But phosphorescence will not appear unless air is admitted into the tube from time to time. This distillation method, however, is not applicable to the quantitative determination of phosphorus in oils, as in phosphorated cod-liver oil, for not more than 36–41 per cent. of the phosphorus will distil over (Stich[1]).

Notes.—As far as loss of phosphorus in phosphorated oils is concerned, the effect of air, light and rancidity is insignificant, at least during the usual period of its use. According to Stich, more dilute phosphorated oils, about 1:1000, keep longer even than those that are more concentrated (1:100).

Quantitative Estimation of Phosphorus in Phosphorated Oil and Phosphorated Cod-liver Oil

1. Method of Enell[2]

In presence of water white phosphorus reacts with iodine in the following manner:

$$\text{(a)}\quad P + 3I = PI_3$$
$$\text{(b)}\quad PI_3 + 3H_2O = 3HI + H_3PO_3$$

Since phosphorous acid is dibasic, 5 molecules of sodium hydroxide are required for the two acids ($3HI$ and H_3PO_3) formed in the reaction from 1 atom of phosphorus (31). Or 1 gram-mol. of sodium hydroxide corresponds to $\frac{1}{5}$ gram-atom $= 3\frac{1}{5} = 6.2$ of phosphorus. Consequently 1000 cc. of 0.1 n-sodium hydroxide solution = 0.62

[1] K. Stich: The Phosphorated Oil Question. Wiener klinische Wochenschrift 14 (1901), 177 and Pharmazeutische Zeitung 47 (1902), 500.

[2] H. Enell: Quantitative Estimation of Phosphorus in Phosphorated Oil. Pharmazeutische Zeitung 50 (1905), 601.

gram of phosphorus, or 1 cc. of 0.1 n-sodium hydroxide solution = 0.00062 gram of phosphorus.

Enell has shown experimentally that iodine has no action upon almond oil under the conditions given below. For example, when 5 grams of this oil, dissolved in 10 cc. of alcohol and 20 cc. of ether, were shaken continuously for 5 minutes with 10 cc. of 0.1 n-iodine solution, iodine had no action upon the oil. Inasmuch as freshly prepared phosphorated oil, but more particularly older oil, always contains phosphorous acid, a test was also made to determine the effect of iodine upon this acid. When 10 cc. of 0.7 per cent. aqueous solution of this acid, both by itself and mixed with 10 cc. of alcohol and 20 cc. of ether, were shaken with iodine for 10 minutes, no iodine disappeared. Enell first determines the acidity of the phosphorated oil. Then in a second test he converts the phosphorus in the oil into phosphorous acid through the tri-iodide and, after removing excess of iodine with sodium thiosulphate, redetermines the acidity. The percentage of phosphorus is finally calculated from the difference between the two titrations, based of course upon equal weights of the phosphorated oil.

Procedure.—Weigh accurately about 1 gram of phosphorated oil and dissolve in a glass-stoppered flask in a mixture of 10 cc. of alcohol and 20 cc. of ether. Add 12 cc. of 0.1 n-iodine solution and shake the mixture for 5 minutes. Exactly remove excess of iodine with 0.1 n-thiosulphate solution which requires vigorous agitation. At once add a drop of phenol-phthalein solution and titrate with 0.1 n-sodium hydroxide solution, until the red color of the mixture after shaking does not increase upon addition of 2 drops more of 0.1 n-sodium hydroxide solution. From the first appearance of rose color until the reaction is completed, usually 0.3 cc. of 0.1 n-sodium hydroxide solution must be added.

Now weigh accurately a second 1 gram portion of phosphorated oil and dissolve it as before in a mixture of 10 cc. of alcohol and 20 cc. of ether. In order that the conditions of titration may be uniform, add 30 cc. of water and a drop of phenol-phthalein solution. Then titrate the acidity as before with 0.1 n-sodium hydroxide solution. In the case of almond oil appearance of a distinct rose color is accepted as the end of titration.

Calculation.—The number of cc. of 0.1 n-sodium hydroxide solution used in the two titrations is calculated in each case for the same weight of oil, most conven-

iently for 1 gram. The difference between the two titrations multiplied by 0.00062 gives in grams the amount of elementary phosphorus in the oil.

Example.—If it is desired to determine also the amount of phosphorous acid, which according to Enell is always formed in the preparation of a phosphorated oil, then it is necessary to determine first the acidity of the oil itself and second the acidity after phosphorus has been dissolved.

Almond Oil: Acidity of 1 gram of oil = 0.686 cc. n-NaOH
Phosphorated Oil: Acidity of 1 gram of oil = 1.36 cc. n-NaOH
= Total acidity before treatment with iodine.

Difference: 1.36 − 0.686 = 0.674 cc. of 0.1 n-sodium hydroxide solution corresponds to the amount of phosphorous acid present. 1000 cc. of 0.1 n-NaOH correspond to $\frac{H_3PO_3}{20} = \frac{82}{20} = 4.1$ grams of H_3PO_3, or $\frac{P}{20} = \frac{31}{20} = 1.55$ gram of phosphorus, or 1 cc. of 0.1 n-NaOH = 0.0041 gram of H_3PO_3, or = 0.00155 gram of phosphorus.

The above difference of 0.674 corresponds to 0.674 × 0.0041 × 100 = 0.2764 per cent. of H_3PO_3, or 0.674 × 0.00155 × 100 = 0.1044 per cent. of phosphorus.

Phosphorated oil after treatment with iodine. Acidity of 1 gram of oil: 15.84 cc. of 0.1 n-NaOH. Difference: 15.84 − 1.36 = 14.48 cc. of 0.1 n-NaOH. This difference corresponds to the amount of free phosphorus = 14.48 × 0.00062 × 100 = 0.898 per cent. of phosphorus. To this must be added 0.1044 per cent. of phosphorus dissolved in the phosphorated oil in the form of phosphorous acid.

Attention is called to the fact that 4–6 per cent. of the phosphorus used in the preparation of a phosphorated oil is oxidized to phosphorous acid.

2. Method of Katz[1]

Katz adopted Straub's[2] method, whereby phosphorus is converted into copper phosphide. He made the observation, however, that the latter is oxidized very slowly. But addition of hydrogen peroxide, especially in presence of ether, greatly increases the speed of the reaction. Another improvement in the method consists in substituting copper nitrate for sulphate, for then phosphoric acid may be directly precipitated with magnesia mixture. Katz takes the following reactions as the basis of the formation of cuprous phosphide, Cu_2P_2, and from that of phosphoric acid:

$$14P + 14Cu(NO_3)_2 + 14H_2O = 7Cu_2P_2 + 28HNO_3 + 14O$$
$$14O + 2Cu_2P_2 + 8HNO_3 + 2H_2O = 4H_3PO_4 + 4Cu(NO_3)_2$$

Procedure.—Agitate vigorously in a separating funnel 10 grams of phosphorated oil with 20 cc. of 5 per cent. copper nitrate solution

[1] J. Katz: Quantitative Determination of Phosphorus in Phosphorated Oil and Similar Preparations. Archiv d. Pharmaz. 242 (1904), 121.

[2] W. Straub: A New Method for the Quantitative Determination of Phosphorus in Oily Solutions. Archiv der Pharmazie 241 (1903), 335.

until a permanent black emulsion is produced. Then add 50 cc. of ether and hydrogen peroxide solution (3 per cent.) free from phosphoric acid in small portions, using 10 cc. in all or sufficient to cause complete disappearance of the black color after vigorous agitation. Separate the water from the ether solution and shake the latter with three separate portions of 10–20 cc. of water. Add a few drops of hydrochloric acid to the combined aqueous extracts and concentrate to 10 cc. upon the water-bath. After further addition of a little hydrochloric acid, filter the liquid to remove oil-drops that have separated. Add sufficient ammonia to the filtrate to cause the precipitate that first forms to redissolve. Finally precipitate phosphoric acid with magnesia mixture and weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

Notes.—Phosphoric acid is often added to commercial hydrogen peroxide as a preservative. For that reason it should be tested with calcium chloride solution before being used. No change should take place. The Katz method involves the following possible sources of error:

1. Presence of phosphorous acid in phosphorated oil, even when freshly prepared, is not taken into consideration (see Enell's method).

2. Oxidation of phosphorous acid is incomplete.

3. Phosphoric acid already in the oil is determined together with that formed by oxidation.

Notwithstanding these defects of the Katz method, it is preferable to the Enell method in case of very weak phosphorated oils, 1:1000 or even weaker. It cannot be used, however, for alcoholic or ether solutions (Katz).

Katz determined the amount of phosphorus in several saturated phosphorated oils and found that on the average they would dissolve at room temperature the following quantities:

Oil	Per Cent of P	Oil	Per Cent of P
Almond Oil	1.13	Cod Liver Oil	1.13–1.25
Olive Oil	1.085	Poppy Oil	1.11
Sesame Oil	1.06	Linseed Oil	1.15
Peanut Oil	1.2	Castor Oil	0.7
Rape-seed Oil	1.16	Paraffine Oil	1.33

3. Method of Fränkel[1] and Stich[2]

In the method of Louise[3] an acetone solution of the phosphorated oil is titrated with a 10 and 1 per cent. silver nitrate solution, until

[1] A. Fränkel: Quantitative Determination of Free Phosphorus in Phosphorated Oils. Pharmazeutische Post 34 (1901), 117.

[2] C. Stich: The Phosphorated Oil Question. Pharmazeutische Zeitung 47 (1902), 500.

[3] E. Louise: Detection and Determination of Free Phosphorus in Oils and Fats. Compt. rend. de l'Acad. des sciences 129 (1899), 394.

a black precipitate of silver phosphide ceases to form. Reduction of silver nitrate solution may be caused by aldehyde in acetone (commercial acetone frequently contains aldehyde), also by phosphorous acid, and finally by certain other substances present in oils, particularly in cod-liver oil. Consequently Fränkel is right in considering the Louise method of determining phosphorus on the basis of silver nitrate solution used as inaccurate. For this reason he causes precipitation by adding hot alcoholic silver nitrate solution to an ether solution of the phosphorated oil and then determines phosphorus in the precipitate as phosphoric acid.

Procedure.—The method follows the specifications given by Bohrisch.[1] Weigh accurately 3–5 grams of phosphorated oil and dissolve in 20 cc. of pure acetone. Pour this solution into 100 cc. of a freshly prepared acetone silver nitrate solution containing 12 drops of 50 per cent. silver nitrate solution. At once shake the mixture vigorously for 5 minutes. Allow to stand for 12 hours in the dark, collect the precipitate upon an ash-free filter, and wash quickly with a little ether-acetone mixture. When most of the alcohol has evaporated, transfer the filter containing the silver phosphide to a boiling-flask, and add 20 cc. of 25 per cent. nitric acid. A few minutes later add 30 drops of fuming nitric acid, close the flask, and set the mixture aside for some time. Open the flask and warm upon the water-bath until the mixture is colorless. Dilute with water, filter and wash with hot water. Warm the filtrate to about 70°, precipitate silver with hydrochloric acid, filter and evaporate the filtrate upon the water-bath to about 5 cc. Finally precipitate phosphoric acid in the usual way with clear magnesia mixture and weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

[1] P. Bohrisch: Phosphorated Oils. Pharmazeutische Zentralhalle 50 (1919), 19.

DETECTION AND ESTIMATION OF SMALL QUANTITIES OF ARSENIC

Destruction of Organic Matter and Detection of Minute Quantities of Arsenic in Organic Substances

(Lockemann[1])

After a critical examination of methods and apparatus proposed for detecting arsenic, Lockemann has come to the conclusion that the most reliable and accurate method is that of Marsh. By means of this method 0.001 mg. of arsenic trioxide may be detected with certainty.

In ordinary toxicological practice it is a question in most instances of detecting quantities of arsenic that are at least injurious to health or possibly fatal in action. In such cases chemicals supplied by reliable manufacturers and guaranteed to be arsenic-free are sufficient. They are adequate when the material is examined for arsenic by one of the less delicate methods.

Lockemann's method of destroying organic matter consists in treating the material with a few cc. of a mixture of 9 parts of fuming nitric acid and 1 part of concentrated sulphuric acid (acid treatment). This treatment is followed by fusion with potassium sodium nitrate (nitrate fusion). Thus all organic matter is completely destroyed and any arsenic is wholly converted into potassium arsenate which may be quantitatively precipitated from aqueous solution with ferric hydroxide. Finally this precipitate is dissolved in sulphuric acid and tested for arsenic in the Marsh apparatus.

Procedure.—After comparing all methods known, Lockemann advises the following procedure for destroying organic matter prior to testing for arsenic. Place the material to be tested for arsenic in a porcelain dish and pour over it a mixture of fuming nitric acid and concentrated sulphuric acid (100:5–10). It is advisable to add the acid mixture in small portions, 1–2 cc. at a time as soon as the reaction subsides. Usually 20 grams of material require 10–20 cc. of the acid mixture. Then apply heat upon the water-bath until the thick mass which is yellow at first becomes brown. Add 30 grams

[1] G. Lockemann: Detection of Small Quantities of Arsenic in Urine, Blood and Other Substances. Biochem. Zeitschr. 35 (1911), 478; The Catalytic Decomposition of Arsine. Zeitschr. f. angew. Chem. 18 (1905), 491; Detection of Arsenic with the Marsh Apparatus. Ibid. 18 (1905), 416; The Evolution of Hydrogen in the Marsh Apparatus. Ibid. 19 (1906), 1362.

of a mixture of equal parts of potassium and sodium nitrate and evaporate to dryness. Yellow crystals are produced as a result of this evaporation. To carry destruction of organic matter farther, the residue is introduced in small quantities into fused potassium and sodium nitrate in a platinum crucible very carefully heated with a small flame. Finally the reaction is brought to an end by applying stronger heat.

APPLICATION OF THIS METHOD TO SPECIAL CASES

(a) Treatment With Acid and Fusion With Nitrate

Urine.—Measure the volume of urine passed during one day and add to a suitable quantity 2.5–3.5 per cent. of a mixture of equal parts of potassium and sodium nitrate. Evaporate this mixture upon the water-bath in a porcelain dish that is not too large, filling it as evaporation proceeds. In case of liquids it is an advantage to add nitrate beforehand, for then the dry residue contains organic matter in very intimate contact with nitrate. While the residue is still moist, add to it under a hood provided with a good draught a mixture of 9 parts of fuming nitric acid and 1 part of concentrated sulphuric acid (acid mixture). Add this acid mixture from a dropping funnel drop by drop, distributing it as uniformly as possible over the entire contents of the dish. The quantity of acid required should be about 1 per cent. of the volume of urine taken. This treatment produces a yellowish brown residue which is then ready for fusion with nitrate. Fuse in a dish, or crucible (platinum or porcelain), over as small a flame as possible 5–10 grams of purified potassium and sodium nitrate. Introduce in small portions, using a platinum spatula, the residue left by evaporation following treatment with acid. After each addition the melt will swell up. As action ceases it will subside. This procedure requires patience but finally all the material will be in the fusion mixture and organic matter will be completely oxidized.

Blood.—Due to presence of haemoglobin and other organic substances, the percentage of protein in blood is high. Consequently complete destruction of blood requires more nitrate. Take 0.75–1 part of solid nitrate mixture for 1 part of blood. Blood serum requires somewhat less, 0.5–0.75 parts of this mixture. Add the necessary quantity of nitrate mixture to the given blood or serum, stir constantly and evaporate in a porcelain dish upon the water-

bath. Carefully add the acid mixture drop by drop before the mixture of blood and nitrate is entirely dry, the volume of which is 10–20 per cent. of that of the blood or serum. Distribute the acid as uniformly as possible over the entire mass. The reaction taking place is rather vigorous and as a result the mass swells up and loses color. The procedure in the nitrate fusion is the same as that described for urine. Iron from haemoglobin separates as red-brown oxide in the under part of the melt.

Parts of Organs and Flesh.—Comminute the material as finely as possible and add for 1 part of the given organ about 0.5 part of acid mixture. The material is placed in a porcelain dish upon the water-bath and the acid mixture gradually added. As a result of this treatment, a thick fluid yellowish mass is obtained. If the acid mixture is added too quickly, carbonization is liable to take place even with evolution of smoke. Mix well the residue from acid treatment by stirring with 1.25–1.5 parts of nitrate mixture based upon the weight of the original material. Evaporation of this mixture will yield a yellowish crystalline residue. The nitrate fusion is carried out in the manner described for blood and urine.

(b) Separation of Arsenic

Transfer the nitrate melt to a beaker with water. If a platinum vessel was used for the fusion, set it while still warm in cold water. The melt will solidify and easily separate from the walls of the container. Heat will then readily bring it into solution. Stir and gradually add from a graduate about 20 per cent. sulphuric acid as long as carbon dioxide and oxides of nitrogen are given off and until litmus paper shows a faint acid reaction. Expel gases completely with heat, cool, add a few drops of methyl orange, and then neutralize with ammonia. Since the adsorptive action of ferric hydroxide for arsenic increases with lowering of temperature, the solution should be cooled with ice. Then add from a burette 20–25 cc. of "iron solution" (page 492). Mix thoroughly and, while stirring constantly, add the same volume of standard "ammonia solution" (page 492). In the case of urine and flesh, most of the iron in the first precipitation is separated as light yellow ferric phosphate. This will influence the adsorptive power of iron for arsenic and arsenic will frequently be found in the second precipitation. After 30 minutes collect the iron precipitate upon paper and wash with cold water to remove adhering nitrate soluton. This should be

continued until the filtrate gives no test for nitric acid with diphenylamine-sulphuric acid. Collect the wash-water by itself and evaporate upon the water-bath. Then add what remains to the main solution before the second precipitation. Or all the wash-waters from the first and subsequent precipitations may be evaporated together and added to the main solution just before the final precipitation. Precipitation with iron should be repeated until the last precipitate proves to be nearly free from arsenic. Starting with 500 cc. of urine, Lockemann recommends using successively 25, 15, 10 and 5 cc. of each reagent, iron and ammonia solution, making four precipitations in all. Ten cc. of iron solution, corresponding to 500 mg. of $Fe(OH)_3$, are sufficient for the complete adsorption at 25° of about 25 mg. of arsenic from 100 cc. of solution, or about 35 mg. of arsenic at 0°. After being washed with cold water (see above), the individual precipitates are dissolved in hot 20 per cent. sulphuric acid and these solutions are tested for arsenic in the Marsh apparatus.

(c) Detection of Arsenic

The form of Marsh apparatus described by Lockemann (Fig. 40) may be conveniently used for this purpose. The procedure carefully avoids passing the gas through cotton or similar material. The gas is dried by means of pure crystallized calcium chloride ($CaCl_2.6H_2O$), since filimentary materials, as well as granulated or fused calcium chloride, cause decomposition of arsine. Adoption of these precautions and introduction of other experimental conditions have increased the sensitiveness of this arsenic test to 0.0001 mg. $= \frac{1}{10{,}000}$ mg. of arsenic. If many arsenic tests are to be made, it is well to have several sets of apparatus. A single ring-stand is sufficient for two sets, one on either side. Removal of air may be hastened by using hydrogen from one Kipp generator. The best metal to use in this apparatus is a zinc-copper alloy, containing 90 per cent. of zinc and 10 per cent. of copper. Hydrochloric acid meeting forensic requirements should be used. Zinc having such a high copper-content has the property of retaining arsenic so that arsenic-free hydrogen is obtained even though the hydrochloric acid may contain a slight trace of arsenic. Hydrogen is washed with water or with dilute sodium carbonate solution. On the other hand, if ordinary zinc and ordinary pure hydrochloric acid are used, hydrogen should first be passed through two wash-bottles containing a

solution of potassium permanganate as concentrated as possible. Thereby arsine evolved together with hydrogen will be oxidized and prevented from entering the Marsh apparatus. The second wash-bottle may be provided with a Y-tube to distribute hydrogen to the two sets of Marsh apparatus side by side. This double current of hydrogen may be regulated by means of pinch-cocks.

Procedure.—Put in the evolution-flask (a) of the Marsh apparatus 5–6 pieces of coppered zinc. Use for this purpose rod zinc (guaranteed arsenic-free) that has been broken into small pieces.

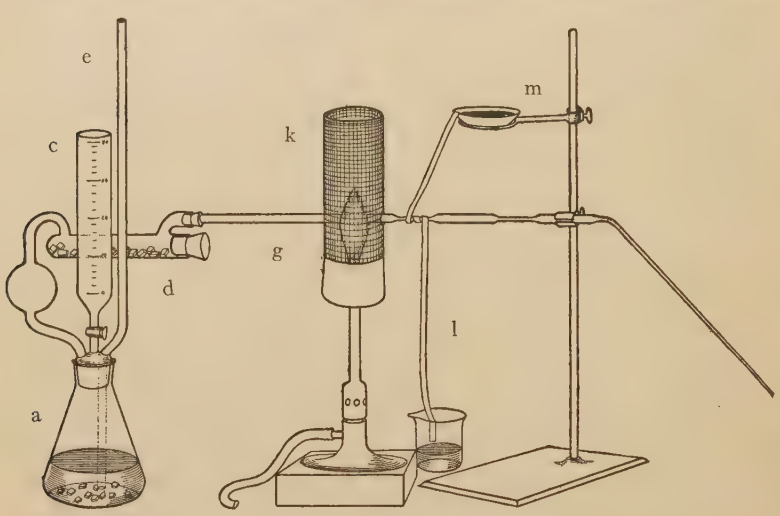


FIG. 40.—Lockemann's Marsh apparatus for detection of arsenic.

Shake these for about 1 minute in a 0.5 per cent. solution of copper sulphate and then rinse several times with water. Close the apparatus and run in 10 cc. of water from the dropping-funnel (c) provided with a glass stop-cock to seal the lower end of tube (e). Attach the ignition-tube (g) to the drying-tube (d) by means of a rubber stopper and clamp firmly at the other end. Connect tube (e) with a Kipp hydrogen generator by means of rubber tubing and open the cock of this Kipp to make sure that the Marsh apparatus is perfectly tight. If the apparatus stands this test, break off the tip of the ignition-tube and pass hydrogen through the system for about half an hour. After that, run into the evolution-flask from the dropping-funnel (c) 10 cc. of 40 per cent. sulphuric acid to generate hydrogen.

The rubber tubing is removed from the tube (e) and the apparatus is ready for use. Light the hydrogen and regulate its flow. Adjust the protective wire mantle (k), wind the moist string (l) 2–3 times around the constricted part of the ignition-tube, and fill the upper dish (m) with water and ice. If after some time neither water nor an arsenic mirror has appeared in the cooled area, add a measured volume of the solution to be tested ($\frac{1}{10}$ or $\frac{1}{4}$) to the dropping-funnel and allow it to run into the apparatus, rinsing with 20 per cent. sulphuric acid. If a Kipp hydrogen generator is not used, begin by running 20 per cent. sulphuric acid into the evolution-flask (a) and wait at least 45 minutes before lighting the hydrogen. Stop the experiment after 2 hours, controlling the evolution of gas during that time, if necessary, by adding more sulphuric acid. Compare arsenic mirrors with the scale of standard mirrors prepared by using known quantities of arsenic. The estimation is more accurate when the quantity of arsenic is very small. It may possibly be carried as high as 12–15 mg. of arsenic.

Activating Agents and Arsenic-free Chemicals

Activating Agents.—In detecting arsenic in the Marsh apparatus, evolution of hydrogen should be hastened by means of an activating agent. Otherwise the reaction proceeds slowly on account of the extreme purity of metal and acid. Platinum chloride and copper sulphate are good activators and have been in use for a long time. Lockemann regards copper sulphate as the best activator because the Marsh test is most sensitive when it is used. But zinc should be coppered before being put into the apparatus and very carefully washed to remove every trace of adhering copper solution. Otherwise copper salt coming in contact with the solution containing arsenic will cause retention of an appreciable part of the arsenic. Platinum chloride behaves in a similar manner. Copper the zinc by putting small pieces, each weighing about 1.2–1.8 grams, in a porcelain dish with dilute copper sulphate solution (1 part of copper sulphate, purified by repeated crystallization, dissolved in 200 parts of water). Shake the pieces of zinc around for about a minute and then rinse several times with water. Dry this coppered zinc upon filter paper and keep in a closed bottle. Using zinc prepared in this manner, Lockemann obtained a distinct mirror even from 0.0001 mg. of arsenic; whereas he was able to detect only 0.001 mg. of arsenic with platinized zinc.

Arsenic-free Reagents.—To prepare salts absolutely free from arsenic, such as sodium chloride, potassium chlorate, sodium and potassium nitrate, add to a concentrated solution of the given salt a ferric salt together with aqueous ammonia without warming. All arsenic present will be precipitated in conjunction with ferric hydroxide. Ammonia itself may also be freed from every trace of arsenic by standing with ferric hydroxide. Since this result is due to adsorption, a little arsenic to be completely precipitated with ferric hydroxide requires considerable ferric salt.

Since sulphuric acid is prepared almost exclusively from crude materials containing arsenic, both the acid and its salts, as well as other preparations depending upon its use, contain a certain amount of arsenic. Sulphuric acid made by the contact process also appears not to be absolutely free from arsenic. When, however, it is a matter of finding quantities of arsenic that are injurious to health, or even produce fatal results, as usually is the case in forensic-chemical examinations, "guaranteed arsenic-free hydrochloric or sulphuric acid" may be used. Furthermore such preparations as a rule are found to be arsenic-free by the less sensitive tests. By passing hydrochloric acid gas through concentrated sulphuric acid, arsenic may be completely removed as trichloride. Such sulphuric acid may be used in preparing arsenic-free hydrochloric and nitric acid from sodium chloride or nitrate rendered absolutely arsenic-free by the above method. Usually sodium and potassium nitrate also contain traces of arsenic. But when examinations dealing with minute traces of arsenic are made, every chemical used should be freed absolutely of the last trace of arsenic by the "iron precipitation method."

Iron Solution.—Lockemann prepares the required "Iron Solution" by dissolving 226 grams of ammonium ferric alum, $(H_4N)Fe(SO_4)_2.12H_2O$, in water, bringing the volume to 1000 cc. 1 cc. of this solution corresponds to 50 mg. of $Fe(OH)_3$.

Ammonia Solution.—This solution is of such concentration that a given volume will precipitate the same volume of iron solution. Adsorption of arsenic by ferric hydroxide proceeds best when the exact stochiometric quantities of ammonia and iron are used. An excess of precipitant is a disadvantage. A solution containing 23.2 grams of ammonia in 1000 cc., that is, a 1.404 n-ammonia is the equivalent of the above iron solution. Since some loss of ammonia by volatilization takes place on standing, it is advisable to use 10 per cent. ammonia and dilute it with four times the quantity of water. Such a solution will be about 2.5 per cent. or 1.47 n-ammonia. This should be titrated with n-acid, using litmus tincture or methyl orange as indicator. If 10 cc. of this ammonia solution requires about 14.5 cc. of n-acid, the adjustment of the solution is correct. Since ammonia solution always contains arsenic, though in very small quantity, one of two procedures should be adopted. The 10 per cent. ammonia may be shaken for some time with freshly precipitated and washed ferric hydroxide, or the solution may be allowed to stand continuously over ferric hydroxide. In the latter case the solution should be shaken from time to time and the required volume filtered off as needed. These solutions of iron and ammonia are used not only to separate arsenic for detection but also for determination.

Purification of Potassium and Sodium Nitrate

Dissolve 500 grams of sodium nitrate in 650 cc. of water and 500 grams of potassium nitrate in 3000 cc. Add to each 25 grams of iron solution, cool in ice-water to about 5°, and precipitate ferric hydroxide by adding 25 cc. of ammonia solution. Allow to stand for a short time and then filter through a plaited paper. Again treat the filtrate in the same manner, using 10 cc. each of iron and ammonia solution.

The second ferric hydroxide precipitate is used to prove whether the nitrate solutions are now arsenic-free or not. Wash it upon the filter with cold, nitrate-free water until the wash-water fails to give the diphenylamine test. Then dis-

solve the precipitate in about 25 cc. of hot 20 per cent. sulphuric acid, cool and test this solution for arsenic in the Marsh apparatus. Should this test show arsenic, the nitrate solutions must be treated again with the corresponding quantities of iron and ammonia solution until a test for arsenic is not given. Ferric hydroxide may be precipitated at ordinary temperature but cooling by means of ice increases adsorption. When the nitrate solutions are free from arsenic, about half of each solution is kept as such. The remainder is evaporated upon the water-bath to get the solid salts. When most of the salt has separated, cool and dry in air between several thicknesses of filter-paper. Mix equal parts of the dry salts. This nitrate mixture is kept in a bottle for use in nitrate fusions.

Notes.—The reagent diphenylamine-sulphuric acid is a solution of 1 part of diphenylamine in 100 parts of concentrated sulphuric acid. The advantage of Lockemann's method of testing for minute traces of arsenic over all other methods is that it does not use large quantities of acid in destroying organic matter. Although the quantity of acid is comparatively small, the quantity of salts is large. But the latter may be completely freed from arsenic with relative ease. Sulphuric and fuming nitric acid required for Lockemann's method, the nitrate fusion method, usually are arsenic-free in the quantities used, that is, a few cc., provided the purest acids of reliable manufacturers are selected.

Incineration Method of Detecting Arsenic

(Strzyzowski[1])

This method is based upon the fact that foodstuffs and animal material containing arsenic yield magnesium pyro-arsenate ($Mg_2As_2O_7$) in the residue obtained by igniting the material with arsenic-free magnesium oxide. Apparently no loss of arsenic occurs during incineration. The residue dissolved in dilute sulphuric acid may be tested for arsenic in the Marsh apparatus.

Procedure.—Put 1 gram of magnesium oxide in a 25 cc. porcelain crucible and add 10 cc. of a liquid, 5–10 cc. of a semi-liquid, or 1 gram of a solid that has been well comminuted. In the latter case, add also 10 cc. of water and in any case 0.5–1 cc. of concentrated nitric acid. The mass should first be dried upon a water-bath or asbestos plate. Then gradually increase the heat over a free flame until the residue, which may have to be broken up, has become pure white. Dissolve the residue in 10 cc. of water and 5.5 cc. of pure, arsenic-free 50 per cent. sulphuric acid. Filter to remove any calcium sulphate that may have separated, washing the residue on the paper with 12.5 per cent. sulphuric acid until the volume of the filtrate is 20–25 cc. Finally test for arsenic in the Marsh appa-

[1] C. Strzyzowski: A Simple Method of Ashing for Rapid Determination of Arsenic in Foodstuffs and Animal Materials. Pharmaz. Post 39 (1906), 977.

ratus. The form of apparatus devised by Strzyzowski has given very good results.

Notes.—The application of this method by the author to the examination of 5 cc. of blood taken from a patient, who had received an intravenous injection of 0.45 gram of neo-salvarsan 1¾ hours before the blood was removed, gave a strong arsenic mirror corresponding to about 0.1 mg. of arsenic. Since the neo-salvarsan contained 30 per cent. of arsenic, the quantity injected represented 0.15 gram or 150 mg. of arsenic. On the basis of a volume of blood in a male adult of about 5 liters, the 5 cc. of blood used in the test could not have contained more than 0.15 mg. of arsenic. The author used in this test a simple form of Marsh apparatus.

Detection of Arsenic in Strzyzowski's[1] Marsh Apparatus

The purpose of this method is to increase sensitiveness by reducing the size of the apparatus, excluding the drying-tube, and doing away with rubber stoppers. The procedure is simpler because the ignition or reduction-tube may be heated without danger of explosion immediately after addition of sulphuric acid, or even before that. This is made possible by passing through the apparatus a current of carbon dioxide dried by means of sulphuric acid.

Apparatus.—This is shown in Fig. 41. Evolution of gas takes place in a vertical tube holding about 40 cc. (a). This is closed by a well-ground, tightly fitting glass cap (b) provided with a side-tube bent at a right-angle. The bottom of (a) is connected with a funnel-tube (d) opening above. This extends at (e) a few mm. into (a) at which point the bore is reduced to 0.5 mm. The cover (f) carrying the entrance-tube (g) is tightly ground into the funnel-tube (d), the internal diameter of which should be at least 5 mm. A current of dry carbon dioxide is passed through (g). The complete apparatus, evolution-tube and funnel-tube, is firmly held by a stopper in a heavy, wide-neck Erlenmeyer flask (h) and surrounded with cold water. The horizontal end of tube (c) is attached by a rubber connector to the reduction-tube (i) made of difficultly fusible glass. The latter should be 15–16 cm. in length of which 4–6 cm. is drawn out to a capillary. This is supported at (j) by a two-pronged fork attached to the first ring-stand. Under the reduction-tube (i) is a gas burner (l) held by the same ring-stand. This is provided with a regulating screw (m) and a chimney (n), the reduction-tube passing through openings cut out of the latter. A porcelain crucible cover (o) is suspended over the chimney and acts as a reflector to concentrate the heat of the flame upon the ignition-tube. The top of the flame should extend a few mm. above the ignition-tube. The flame used in detecting quantities of arsenic under 0.05 mg. should be free from carbon. The capillary tip of the reduction-tube (i) extends several mm. into the reduced end of the suction-tube (p). The bore of this reduced end should always be somewhat larger than that of the capillary end of tube (i). This

[1] C. Strzyzowski: An Improvement in the Marsh Apparatus and a Method of Recognizing Arsenic in Minute Deposits of Arsenic. Oesterr. Chemiker Ztg. 7 (1904), 77.

part of suction-tube (p) is held by a clamp attached to the middle ring-stand (q). Suction-tube (p) leads to a catch-bottle (r), holding about 50 cc. and containing a little 3 per cent. silver nitrate solution, and thence to a water-pump. Use of suction makes it possible to remove moisture constantly; also with decreased pressure exit of hydrogen is easy when very fine capillaries are used; and finally the Marsh-Berzelius and Gutzeit tests may be combined. In the latter test a black precipitate of metallic silver separates if a dilute solution of silver nitrate is used. A movable porcelain crucible cover (s), turned toward the eye, is held behind the constricted part of the reduction-tube close to the chimney (n). It facilitates detection of any arsenic mirror that may appear and at the same time may be used to produce arsenic spots. Ignition-tubes (reduction-tubes) should be made of potash glass having a wall-thickness of about 1.5 mm., a bore of 4 mm., and a length of 20 cm. They should be drawn out to a fine capillay in the middle and then halved. If the constriction is not fine enough, it may be drawn out more in

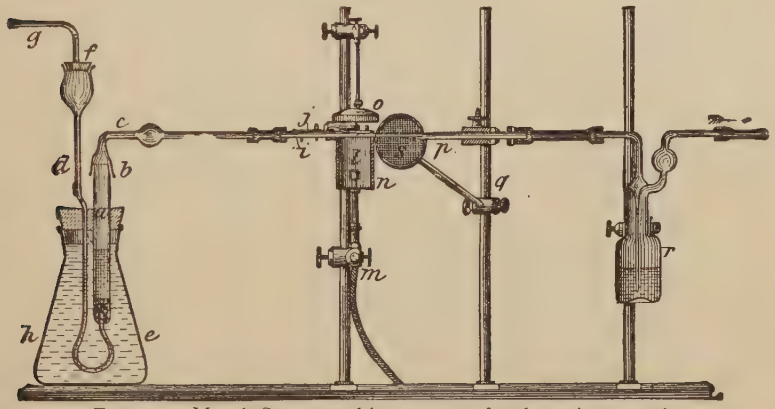


FIG. 41.—Marsh-Strzyzowski apparatus for detecting arsenic.

a small flame until the capillary is finer than the finest needle of a hypodermic syringe and not more than 0.1 mm. in bore. The finer the capillary, the shorter it should be (about 4 cm.), otherwise the suction-pump is unable to overcome the resistance of capillarity. In most tests capillaries of larger diameter may be used.

Procedure.—Use in all cases 10 grams of zinc broken into 10–15 pieces. Two strengths of sulphuric acid are required, one about 50 per cent. (1 : 1) and the other 12.5 per cent. (7 volumes of water to 1 volume of sulphuric acid). As catalyzer Strzyzowski used 1–2 drops of 0.4 per cent. platinic chloride solution. This solution should always be freshly prepared by diluting a 1 per cent. solution that keeps on standing. To test reagent, put 10 grams of zinc in the evolution-tube (a) and place this in flask (h) containing water at 20-25°. Pass a current of carbon dioxide, washed through concentrated sulphuric acid, into the apparatus for 15–30 seconds, using the suction-pump if necessary. Adjust cap (b) after about 30 seconds, push the capillary tip 2 mm. into (p) but not so that (p) fits tightly, and light the burner. Introduce through funnel-tube (d) a drop of the platinum solution and 10 cc. of a cooled mixture of 5 cc. of 50 per cent. sulphuric acid and 15 cc.

of water. By means of the suction-pump regulate the pressure in the apparatus so that the meniscus of the liquid in tube (d) is always lower than that of the liquid in evolution-tube (a). After 15–30 minutes add 10 cc. of dilute sulphuric acid and 1 drop of platinum solution. Rinse the funnel-tube with 5–10 cc. of 12.5 per cent. sulphuric acid and allow the apparatus to run for 2.5 hours. After that time if no film of arsenic can be seen, the reagents are arsenic-free up to 0.0001 mg.! A film that is still visible is given by only 0.0001 mg. of arsenic trioxide. This quantity is considered the extreme limit of arsenic detectable by the Marsh-Strzyzowski procedure.

The procedure in the examination proper is analogous to that above but with this difference that the solution to be tested, in case it contains more than 0.01 mg. of arsenic, is introduced into the apparatus in 3–6 instead of 2 portions, the first portion amounting to 10 cc. If the quantity of arsenic trioxide is less than 0.005 mg., the water in Erlenmeyer flask (h) should be warmed to about 25°. After arsenic has been deposited, if the capillary end is broken off about 3 mm. above the deposit and the latter is heated by a 3 mm. high flame of a microburner applied for 1–2 seconds at the tip, a distinct odor of arsenic may be recognized by holding the capillary at once under the nose.

Notes.—In Strzyzowski's method hydrogen both with and without arsine is not dried before entering the decomposition-tube, that is, reduction-tube (i). The author has found as a result of long years of experience that the sensitiveness of the Marsh test is lessened if the gas is not dried beforehand. The modification proposed by Gadamer of introducing a small drying-tube containing 10–15 drops of concentrated sulphuric acid in tube (c) between evolution-tube (a) and reduction-tube (i) is to be recommended.

Morin and Son in Basel, Switzerland, furnish the apparatus constructed according to the specifications given by Strzyzowski.

ADDITIONAL ARSENIC TESTS

Associated with the Marsh test are certain other quite sensitive tests for arsenic that are of especial importance in toxicological examinations. These are the tests of Gutzeit, Bettendorff and Reinsch, as well as the biological test.

The Gutzeit Test for Arsenic[1]

This arsenic test is based upon the behavior of arsine toward a saturated aqueous solution of silver nitrate (1:1). A yellow molecular compound of silver arsenide and silver nitrate is formed. In contact with water this compound immediately turns black owing to separation of metallic silver:

(a) $6AgNO_3 + AsH_3 = 3HNO_3 + AsAg_3.3AgNO_3$ (yellow)
(b) $AsAg_3.3AgNO_3 + 3H_2O = H_3AsO_3 + 3HNO_3 + 6Ag$ (black).

[1] Gutzeit: Pharm. Ztg. 24 (1879), 263.

Procedure.—Put in a test-tube a piece of zinc, a few cc. of dilute, arsenic-free sulphuric acid, or better hydrochloric acid, and the material to be tested for arsenic. Insert a roll of absorbent cotton in the upper part of the tube and cover the top with a piece of filter-paper moistened with concentrated silver nitrate solution (1:1). If arsenic is present, a lemon-yellow color appears where the paper is moist, turning black on addition of water. Frequently when no water is added, the periphery of the yellow spot soon becomes brownish black. This change gradually extends to the centre and finally the entire spot becomes black. In case of larger quantities of arsenic, particularly if the evolution of gas is too rapid, a fugitive yellow color that quickly turns black is all that is observed.

Another method of making the test is to cover the mouth of the test-tube with a piece of filter-paper and place upon it a crystal of silver nitrate. If arsenic is present, the crystal will first turn yellow and then soon become black.

Notes.—The Gutzeit test is very sensitive, for it permits detection of 0.01 mg. of arsenic. But it is not as characteristic of arsenic as, for example, Bettendorff's test, since phosphine and stibine under the same conditions produce similar colorations with silver nitrate. The former also gives a yellow compound ($PAg_3.3AgNO_3$) that becomes black with water. Even dry hydrogen sulphide will produce a yellow or yellow-green spot upon paper moistened with concentrated silver nitrate solution. This spot has a black margin that gradually extends over the entire area. Poleck and Thümmel[1] have determined the composition of this yellow compound ($Ag_2S.AgNO_3$).

Modifications of the Gutzeit Arsenic Test

(Flückiger,[2] Smith and Beck-Merres[3])

Flückiger was the first to recommend substitution of mercuric chloride for silver nitrate. Several other investigators have also used this salt. Beck and Merres, following the procedure of Smith, used mercuric bromide, since its colorations are more stable than those given by the chloride.

Filter-paper moistened with mercuric chloride solution is first turned yellow by arsine and then brown when action is prolonged.

[1] Th. Poleck and K. Thümmel: Some New Silver Compounds. Ber. d. Deutsch. chem. Ges. 16 (1883), 2435.

[2] F. A. Flückiger: Detection of Minute Quantities of Arsenic. Arch. d. Pharm. 27 (1889), 1.

[3] K. Beck and Merres: Determination of Small Quantities of Arsenic with Special Reference to the Method of Smith. Arb. a. d. Kais. Ges.-Amt. 30 (1915), 38.

The yellow spot, however, is never as bright and strong as that given by silver nitrate. But it is not changed either by light or water. In every instance the yellow as well as the brown spot remains unchanged for the duration of the experiment.

According to Flückiger, concentration of mercuric chloride solution has no marked influence upon the reaction, that is, the coloration on the paper is nearly the same whether saturated or very dilute mercuric chloride solution is used. Alcoholic mercuric chloride solution possesses no advantage over an aqueous solution. Mercuric chloride also behaves exactly like silver nitrate toward phosphine, stibine and hydrogen sulphide. Consequently in both cases hydrides of antimony, phosphorus and sulphur should not be present, if arsenic is to be recognized with certainty by this test. But the mercuric chloride test is to be preferred to that with silver nitrate, or at least given equal consideration, since it is practically unaffected by light and water.

A small quantity of arsine, according to Gotthelf,[1] produces the yellow compound ($AsH(HgCl)_2$) with mercuric chloride and a larger quantity the red-brown compound ($As(HgCl)_3$):

(a) $AsH_3 + 2HgCl_2 = 2HCl + AsH(HgCl)_2$ Yellow
(b) $AsH_3 + 3HgCl_2 = 3HCl + As(HgCl)_3$ Red-brown

In addition to the above compounds, $AsH_2(HgCl)$ and As_2Hg_3 also exist (Partheil[2]).

In these arsenic tests usually the yellow compound alone appears. If the test is to be characteristic of arsenic, hydrides of sulphur, phosphorus and antimony should be excluded. Consequently the material at the outset should be oxidized to convert such compounds as may cause interference into their highest state of oxidation which resists reduction. For this purpose Gotthelf prefers nitric acid to iodine. On the other hand, the arsenic test is sharper, if this element is in the trivalent condition. Therefore Gotthelf after oxidation reduces with sulphur dioxide and then evolves arsine with a little granulated zinc and hydrochloric acid. The gases first pass through cotton impregnated with lead acetate and then act upon the mercuric chloride paper.

[1] A. Gotthelf: The Gutzeit Detection of Arsenic with Mercuric Chloride. Jour. Soc. Chem. Ind. 22 (1902), 191.

[2] A. Partheil: Contribution to the Knowledge of Arsenic Compounds. Arch. d. Pharm. 237 (1899), 121.

Smith[1] has improved this method of substituting mercuric bromide for chloride, Goode and Perkin[2] having previously shown that colorations produced by mercuric bromide are more stable than those given by the chloride. It was also shown that mercuric bromide is more sensitive toward arsine than mercuric chloride. Beck and Merres[3] tested Smith's method and found it sufficiently exact to be used in detecting very small quantities of arsenic such as may occur in foodstuffs.

Quantitative Estimation of Arsenic and Antimony by the Gutzeit Method

Using a special apparatus and paper sensitized with mercuric chloride, Sanger and Black[4] found that the Gutzeit test may be employed to determine small amounts of arsenic quantitatively. The process is very simple and requires only a short time for completion. Sanger and Riegel[5] have extended this method to the estimation of antimony.

Sensitized Paper.—Paper strips[6] uniformly 4 mm. wide are sensitized by being soaked in 5 per cent. solution of recrystallized mercuric chloride. These are dried, cut into 7 cm. lengths, and protected from light and moisture in a stoppered bottle containing calcium chloride, or soda lime, covered with cotton

Apparatus.—A 30 cc. bottle (Fig. 42) for the reduction is closed by a glass stopper provided both with a thistle-tube, constricted to 2 mm. at the end and extending nearly to the bottom of the bottle, and with an exit-tube widened to about 15 mm. just above the stopper. Connected with this exit-tube by a ground joint and at right angle is a tube exactly 4 mm. inside diameter and approximately 9 mm. in length from the bend.

[1] Smith: U. S. Dept. of Agriculture. Bureau of Chemistry. No. 102, Page 5.

[2] J. A. Goode and F. M. Perkin: Observations on the Gutzeit Test for Arsenic. Journ. Soc. Chem. Ind. 25 (1906), 507.

[3] K. Beck and Merres: Determination of Small Quantities of Arsenic with Special Reference to the Method of Smith. Arb. a. d. Kais. Ges.-Amt. 50 (1915), 38.

[4] Sanger and Black: Proc. of the Amer. Acad. of Arts and Sciences 43 (1907), 297.

[5] Sanger and Riegel: Ibid. 45 (1909), 21.

[6] A cold pressed paper made by Whatman has been found to give the best results.

Procedure.—Place 3 grams of uniformly granulated zinc[1] in the bottle and a strip of sensitized paper in the 4 mm. deposition-tube. In estimating arsenic, place in the enlargement of the exit-tube a loose plug of clean absorbent cotton that has been kept over sulphuric acid; an hour's preliminary run is necessary to moisten the cotton partially. In the case of antimony, substitute for cotton a disc of filter-paper that has been moistened with normal lead acetate solution, dried and kept in a well-stoppered bottle. Before inserting this disc, moisten with a drop of water. Next add 15 cc. of diluted hydrochloric acid[2] (1:6) and let the hydrogen run 10 minutes to make sure that the reagents cause no stain. Then add the whole, or an aliquot part of the solution to be tested. Arsenic will produce a color on the paper in a few minutes which will reach a maximum within 30 minutes. Antimony produces no visible effect on the sensitized paper, unless the amount is above 70 mmgr. (= 0.070 mg.) when a gray color may appear. If there is any color, another trial should be made with a smaller portion of solution. In the determination of arsenic, a disc of lead acetate paper should be inserted beneath the cotton as a precaution against possible formation of hydrogen sulphide.

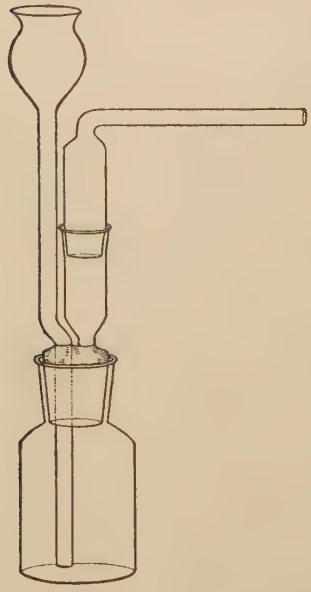


FIG. 42.—Apparatus for the quantitative Gutzeit method.[3]

Standard Bands. (a) **Arsenic.**—Dissolve 1 gram of resublimed arsenious oxide in a little arsenic-free sodium hydroxide, acidify with sulphuric acid, and make up to a liter with recently boiled water. Dilute 10 cc. of this solution (I) to a liter with freshly boiled water which gives solution (II) containing 0.01 mg. of arsenious oxide per cc. Using definite volumes of solution II, measured from a bur-

[1] Bertha spelter from the New Jersey Zinc Company, New York, has been proved free from arsenic.

[2] Synthetic hydrochloric acid, made from electrolytic hydrogen and chlorine by the Hooker Electrochemical Company, New York, is said to be entirely free from arsenic. Tr.

[3] This all-glass apparatus, suggested by W. A. Boughton, is now in use in the Harvard laboratory and is a modification of Sanger's original apparatus.

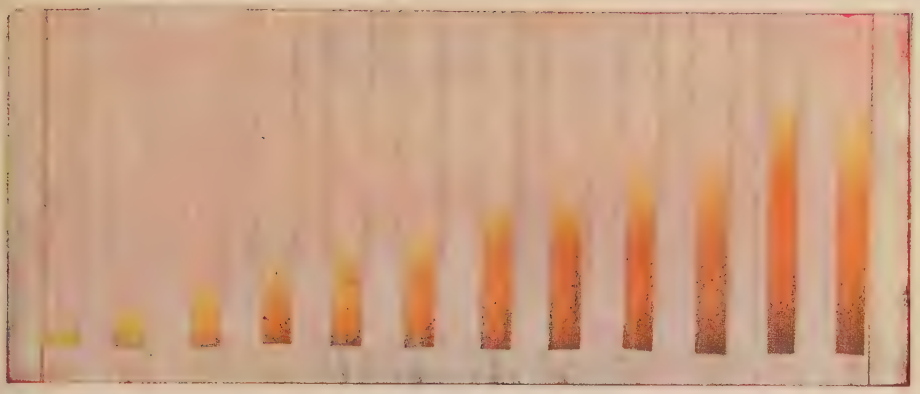


FIG. 43.—Standard arsenic bands in micromilligrams of As_2O_3 (initial).

FIG. 43a.—Standard arsenic bands in micromilligrams of As_2O_3 (hydrochloric acid development).

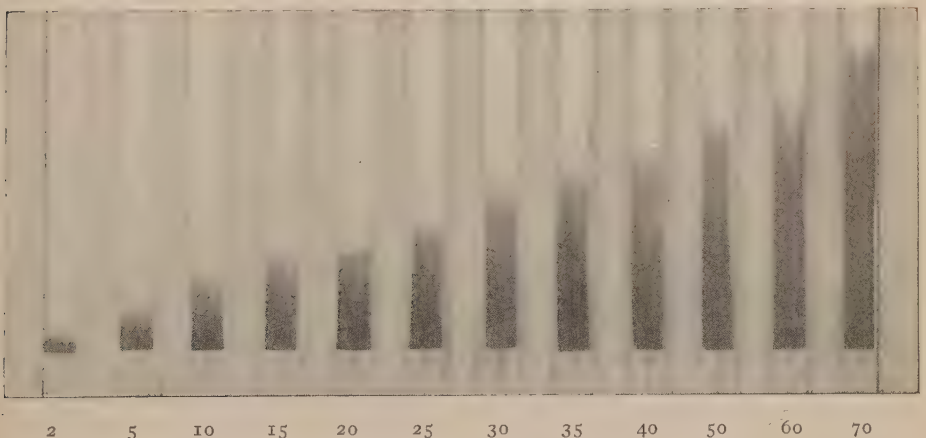


FIG. 43b.—Standard arsenic bands in micromilligrams of As_2O_3 (ammonia development).

(*Facing Page* 500)

ette, prepare a series of color-bands (Fig. 43), taking a fresh charge of zinc and acid for each portion. The color ranges from lemon-yellow through orange-yellow to reddish brown.

(b) **Antimony.**—Dissolve 2.3060 grams of pure, recrystallized tartar emetic in a liter of water. This solution (I) contains 1.0 mg. of antimonious oxide per cc. By dilution of (I), solutions containing 0.01 mg. (II) and 0.001 mg.(III) are prepared and used in making sensitized bands.

These bands eventually will fade but they may be preserved longer by being sealed in glass tubes in the bottom of which is phosphorus pentoxide covered with cotton. The color of the arsenic bands may be developed (1) by placing the band in hydrochloric acid (1:1) for 2 minutes at a temperature not over 60°, washing thoroughly, drying and sealing as before; (2) by treating for a few minutes with ammonium hydroxide, which gives a dense, coal-black color, washing, drying and sealing in a tube over quicklime.

To develop the antimony band, let it stand in a test-tube covered with normal ammonium hydroxide 5 minutes. A black band is slowly developed. These bands may be protected as described, or placed between glass plates cemented together and bound with passepartout paper.

The more dilute standard solutions must be freshly made up within a few hours of use.

Notes.—Solutions should be as free as possible from sulphur compounds yielding hydrogen sulphide; interfering organic matter; and metals retarding formation of arsine and stibine. The cotton in the exit-tube should be replaced after 10–12 runs, and the lead acetate disc after each run. If the solution contains arsenate, reduce with 10 cc. of arsenic-free sulphurous acid and expel the excess.

The absolute delicacy of the method is set at 0.00008 mg. of arsenious oxide and 0.0005 mg. of antimonious oxide. The practical delicacy, using a band 4 mm. wide, is 0.001 mg. of arsenious oxide and 0.002 or 0.003 mg. of antimonious oxide. By using, however, a band 2 mm. wide in a correspondingly narrow exit-tube, a practical delicacy of 0.0005 mg. of arsenious oxide and 0.001 mg. of antimonious oxide is obtainable. In length of band and density of developed color, the effect of arsine on the sensitized paper is from 2–3 times as great as that of stibine. The authors do not claim a greater accuracy for the method than within 10 per cent.

Bettendorff's Test for Arsenic

This test is based upon the fact that compounds containing arsenic are reduced by stannous chloride solution in presence of an excess of strong hydrochloric acid to black arsenic. The reagent used in this test is the "Solutio stanni chlorati" of the German Pharmacopoeia. Hydrochloric acid first converts arsenious oxide and

arsenites into arsenic trichloride and the latter is then reduced by stannous chloride:

$$(a) \quad As_2O_3 + 6HCl = 2AsCl_3 + 3H_2O$$
$$(b) \quad 2AsCl_3 + 3SnCl_2 = 2As + 3SnCl_4$$

In the cold, this reagent reduces arsenic acid very slowly but separation of metallic arsenic takes place quickly with heat. The fourth edition of the German Pharmacopoeia directs that officinal preparations be tested for arsenic by Bettendorff's test. Hydrochloric acid, for example, is tested in the following manner: "Add 3 cc. of stannous chloride solution to 1 cc. of hydrochloric acid. No coloration in the course of 1 hour should appear." Such a mixture containing traces of arsenic is first colored brown to black-brown and, after long standing, deposits black metallic arsenic. Although the Bettendorff test is less sensitive than that of Gutzeit, it possesses the great advantage over the latter of giving no coloration, even after long standing, with substances containing antimony. The Bettendorff reagent behaves toward selenium as toward arsenic.

Contrary to statements of different authorities, Winkler[1] has shown that a test for arsenic may be made in presence of sulphuric acid, or sulphates, without formation of tin sulphide even after warming. Winkler adds 10 cc. of clear, decanted reagent (see below), prepared according to his directions, to 2 cc. of the solution of the material, prepared if possible with hydrochloric acid, and heats the mixture to boiling. M. de Jong (see below) uses an ethereal solution of stannous chloride containing hydrochloric acid. The liquid to be tested for arsenic is mixed with 5 cc. of hydrochloric acid and 5 cc. of the reagent. This mixture is thoroughly shaken and placed for a minute in water warmed to about 40°. If arsenic is present, a brownish red ring appears at the zone of contact of the hydrochloric acid and ether solution. Arsenic in dilution of 1:250,000 still gives a distinct test.

Preparation and Delicacy of Different Modified Bettendorff Reagents (Vanino and Hartwagner[2])

Preparation of Reagent

1. The fifth edition of the German Pharmacopoeia prescribes stirring 5 parts of crystallized stannous chloride ($SnCl_2.2H_2O$) to a magma with 1 part of officinal

[1] L. W. Winkler: Detection of Arsenic by Bettendorff's Reagent. Zeitschr. f angew. Chem. 26 (1913), 143.

[2] L. Vanino and F. Hartwagner: Bettendorff's Reagent and Its Modifications. Arch. d. Pharm. 252 (1914), 381.

hydrochloric acid (sp. gr. 1.126 = 25.7 per cent.) and saturating with dry hydrochloric acid gas. The solution obtained is allowed to settle and is then filtered through asbestos.

2. Immendörffer-Kornthal[1] dissolve 1 part of crystallized stannous chloride in 3 parts of 30 per cent. hydrochloric acid, cool well with ice, and add 1 part of concentrated sulphuric acid. If the materials are absolutely free from arsenic, this mixture should have no color.

3. Warnecke[2] prepares a solution of 1 part of crystallized stannous chloride in 2 parts of strongly fuming hydrochloric acid (sp. gr. 1.19–1.20 = 38 per cent.). The delicacy of this solution is said to be almost as great as that of the Marsh test.

4. Moberger[3] has shown that this reagent loses nothing in sensitiveness, if less stannous chloride is used. If 100 grams of $SnCl_2.2H_2O$ are dissolved in 38 per cent. hydrochloric acid to a volume of 500 cc., the quantity is sufficient.

5. Winkler[4] has taken even half the quantity of stannous chloride used by Moberger, having dissolved 100 grams of $SnCl_2.2H_2O$ to a volume of 1000 cc. in 36–38 per cent. hydrochloric acid. The solution, which is usually slightly brownish (trace of arsenic in the acid), becomes clear after standing for some time. This result may be hastened by allowing the reagent to stand in a warm place, especially if 1 gram of powdered glass is added for a liter of solution. When clear, this reagent is entirely colorless.

Use.—Warm 2 cc. of the solution to be examined for arsenic, prepared if possible with hydrochloric acid, with 10 cc. of the reagent gradually to boiling. Then allow the test to stand for half an hour. Dilute the concentrated sulphuric acid beforehand with an equal volume of water. The limit of delicacy is about 0.001 gram of As_2O_3 in a liter of solution.

6. An ether solution of stannous chloride containing hydrochloric acid is, according to de Jong,[5] a very sensitive reagent for arsenic. Dissolve 25 grams of stannous chloride in 100 cc. of ether and add 20 cc. of hydrochloric acid.

The results obtained by Vanino and Hartwagner have shown that all these modified Bettendorff reagents will detect trivalent and quinquevalent arsenic. In the case of de Jong's reagent, the limit of delicacy is 0.03 mg. of quinquevalent arsenic. This reagent was found to be the most sensitive of all for trivalent arsenic (limit of delicacy 0.0015 mg. of trivalent arsenic). The reagent prescribed by the German Pharmacopoeia was found to be most sensitive for quinquevalent arsenic (limit of delicacy 0.006 mg. of quinquevalent arsenic). The reagent of the German Pharmacopoeia is equally suitable for trivalent and quinquevalent arsenic. The reagents of Warnecke, Moberger and Winkler, because of the ease with which they are prepared and their relatively high sensitiveness, are to be highly recommended. By the latter modification of Bettendorff's reagent a distinct test is given by 0.015 mg. both of trivalent and quinquevalent arsenic.

[1] Immendörffer-Kornthal: Pharm. Zentralbl. 32, 740.

[2] Warnecke: Pharm. Ztg. 1891, 167; Pharm. Zentralhalle 32 (1891), 184.

[3] Moberger: Pharm. Zentralbl. 37 (1896), 199.

[4] L. W. Winkler: Detection of Arsenic by Bettendorff's Reagent. Zeitschr. f. angew. Chem. 26 (1913), 143.

[5] M. de Jong: Stannous Chloride. Zeitschr. f. analyt. Chem. 41 (1902), 596.

Reinsch[1] Test for Arsenic and Special Applications

The principle of the Reinsch test is that arsenic is precipitated by metallic copper from hydrochloric acid solutions as gray copper arsenide, Cu_3As_2:

$$2AsCl_3 + 6Cu = 3CuCl_2 + Cu_3As_2$$

In carrying out this test, add hydrochloric acid to the solution to be tested for arsenic and add a piece of bright sheet copper. If the solution is rather concentrated and contains more than traces of arsenic, the copper will turn gray in the cold. More dilute solutions, however, have to be warmed before separation of arsenic takes place. When the quantity of arsenic is larger, copper arsenide deposited on the copper easily detaches itself. In hydrochloric acid solution, arsenic acid is reduced by copper, at least when heat is applied.

In presence of hydrochloric acid, antimony is also precipitated by copper. Consequently the Reinsch test is characteristic of arsenic only in absence of antimony. A test for arsenic in presence of antimony may be made by drying the gray deposit on the copper, heating and noting whether it develops a garlic-like odor.

The Reinsch test is particularly applicable in examining wall-papers for arsenic. Cut the given paper into as small pieces as possible, cover with hydrochloric acid (1:2), add a clean piece of bright copper, and apply heat. In presence of arsenic the copper will turn gray.

The Reinsch test is also very useful in detecting arsenic quickly in foods and beverages, especially when many tests have to be made. As a result of a great many arsenical poisonings, that occurred at one time in Lancashire and were traced to use of beer containing arsenic, Chapman[2] and Allen[3] both made use of the Reinsch test in a slightly modified form.

The procedure adopted by Chapman consisted in acidifying 500 cc. of beer in a beaker with a few drops of hydrochloric acid and warming upon a sand-bath until most of the alcohol had been expelled. He then added to the residual liquid hydrochloric acid, amounting to about one-fifth of its volume, and introduced a piece of bright copper gauze about 2 cm. square attached to a copper rod. If the copper remained perfectly bright after gentle boiling for 1 hour, the beer was free from arsenic, or contained less than 1 part per million. If, however, the cop-

[1] Reinsch: Journ. f. prakt. Chem. 24 (1841), 244.

[2] A. C. Chapman: Detection of Arsenic in Beer and Brewery Materials. The Analyst 26 (1902). 8.

[3] A. H. Allen: Detection of Arsenic in Beer. The Analyst 26 (1902), 10.

per showed any discoloration, it was removed, carefully rinsed first in running water and then in alcohol and ether, and finally dried at low temperature. The copper was then carefully rolled and introduced into a glass bulb-tube about 7.5 cm. long. The tube was held in a horizontal position and heated at the end to a red glow. Any arsenic present was oxidized and deposited as a crystalline sublimate in the form of a white ring. The octahedrons and tetrahedrons characteristic of arsenic trioxide could be recognized under the microscope.

Chapman tested samples of grape-sugar, invert-sugar, malt and similar materials by dissolving 50 grams in 500 cc. of water, acidifying with 50 cc. of hydrochloric acid, and then proceeding as already described for beer. During the latter part of the heating for 1 hour, these substances usually show charring. In such cases the copper gauze was taken out of the solution sooner at definite intervals. If the copper was found to be discolored after about 15–30 minutes, heating was stopped and the copper was examined as above. Chapman showed that arsenic in beer, responsible for so many poisonings at that time, came from glucose used in brewing the beer.

Allen first tested samples of beer for arsenic in the Marsh apparatus. He found that sulphites in beer acted detrimentally, for hydrogen sulphide was formed by nascent hydrogen and that precipitated arsenic as sulphide. For that reason Allen first oxidized the beer with bromine water, boiled off excess of bromine, and then added the solution together with some cuprous chloride to the reduction-flask of the Marsh apparatus.

Allen also found the Reinsch test very convenient when many examinations had to be made. In this case the precaution was also taken of first removing sulphites that might be present. A volume of about 100 cc. of beer was heated to boiling for a few minutes with hydrochloric acid and some bromine water. A solution of cuprous chloride in hydrochloric acid was then added to reduce any oxidized arsenic to arsenious acid and the solution was boiled with a piece of copper foil 1 cm. square. In other respects the procedure adopted by Allen was like that used by Chapman. A distinct test is said to be given by 1 part of arsenic trioxide in 2,000,000 parts of beer, equivalent to 0.05 mg. of As_2O_3 in 100 cc. of beer.

Biological Detection of Arsenic by Penicillium Brevicaule

Gosio[1] was the first to show that certain moulds, grown upon media containing minute quantities of arsenic, produce volatile arsenic compounds characterized by a garlic-like odor. Seven species of moulds were found to possess this property Penicillium brevicaule, however, which Gosio isolated from air, and which was first found upon decaying paper, possessed this property in highest degree. Gosio states that we are justified in regarding Penicillium brevicaule as a living reagent for arsenic. Even 0.0001 gram of arsenic may be recognized with certainty by this biological test.

[1] R. Gosio: Action of Certain Moulds upon Arsenical Compounds. Rivista d'igiene è sanità publica (1892),201.

The test is so delicate that it should be of great value in toxicological analysis in a preliminary examination for arsenic.

Maasen[1] states that a temperature of 28–32° is most favorable to the growth of the mould. Crumbs of wheat bread were found to make an especially good culture-medium. When this material is used, a vigorous growth of mould is visible even in 48 hours. Sometimes a test for arsenic may be finished in a few hours, and always in 2 or 3 days. The characteristic garlic odor from weak arsenical cultures may be distinctly recognized even after several months. That these "arsenic moulds" do not produce gases having a garlic odor from sulphur, phosphorus, antimony, boron and bismuth compounds, is an important fact. But Penicillium brevicaule possesses in high degree the power of converting solid selenium and tellurium compounds into volatile substances having a peculiar odor. The odor, especially from tellurium cultures, is like that produced by arsenic cultures, namely, distinctly like garlic! The odor from selenium cultures, however, differs from that arising from arsenic cultures. It is more of a mercaptan odor.

Biginelli[2] found that the gases generated from arsenic cultures by Penicillium brevicaule are completely absorbed by mercuric chloride solution. Colorless crystals having the composition $AsH(C_2H_5)_2.2HgCl_2$ are formed. This is a double compound of mercuric chloride and diethyl arsine. This compound is easily decomposed. It then diffuses an intense garlic odor.

Abel and Buttenberg[3] state that a mould to be of use in the biological detection of arsenic must satisfy the following conditions: "It must grow rapidly and not generate any odors during growth, except the garlic odor produced from an arsenical medium. It must not be restricted as to culture-medium. It must grow in presence of large, or very small quantities of arsenic. Finally, it must demonstrate its specific action in presence of metallic arsenic and all kinds of arsenical compounds."

The best material for these experiments is white or Graham bread, either of which is a favorite culture-medium for moulds. The crust

[1] A. Maasen: The Biological Method of Gosio for Detecting Arsenic and Formation of Organic Compounds of Arsenic, Selenium and Tellurium by Moulds and Bacteria. Arb. a. d. Kais. Ges. Amt. 18 (1902), 475.

[2] P. Biginelli: Composition and Chemical Constitution of Arsenical Gases from Wall Papers. Atti. R. Acad. Dei Lincei Roma (5) 9. II (1900), 210.

[3] R. Abel and P. Buttenberg: Action of Moulds upon Arsenic and Its Compounds. Detection of Arsenic Biologically. Zeitschr. f. Hyg. 32 (1899), 449.

is the only part of the bread having a specific aromatic odor. When this has been removed, the crumbs may be said to be practically odorless.

Procedure.—When the material examined is liquid, absorb it completely by adding bread crumbs and scatter a small quantity of dry bread over the surface. Solid material should be finely ground, or cut into as small pieces as possible, and placed in not too small a flask. Add at least the same quantity of bread crumbs, thoroughly mix the two substances by shaking, and moisten the mass with a little water. Close the flask with a cotton plug and sterilize in steam. Sterilization must kill all micro-organisms in the flask. Therefore, heat the flask in an autoclave for 30 minutes under a pressure of 1–1.5 atmospheres. If an autoclave is not available, it may be sterilized in a current of steam 3 times for 1 hour each at intervals of several hours. There is no danger of volatilizing arsenic during sterilization. Then inoculate the sterilized material when cold. Place in a flask a slice of potato, superficially coated with mould in the spore-forming stage, and agitate with bouillon (peptone), salt solution or sterilized water, and make sure that it is as finely broken up as possible by means of a strong platinum wire that has been ignited. Observe all necessary precautions, and add the mould suspended in water in sufficient quantity to impregnate the entire surface of the material suspected of containing arsenic. There should not be more liquid, however, than the culture-medium will absorb. Too much moisture retards growth of the mould. Finally, draw a tight rubber cap over the mouth of the flask and cotton plug. Flasks thus closed may stand in the room, but it is better to keep them at higher temperature, for example, in an incubator at 37°, since these conditions are most favorable to the growth of the mould. As soon as the growth of mould upon the medium is distinctly visible to the naked eye the first indication is given that a test of the culture for volatile arsenic compounds may prove successful. In a very favorable case, this is possible in 24 hours. The growth of mould is always luxuriant in 48–72 hours, so that a decision may be reached. If there is no odor, the flask is closed and the test is repeated once or twice daily on succeeding days. Even in presence of only 0.01 mg. of arsenic, the specific garlic odor is apparent and persists for a considerable time. A fraction of a mg. of arsenic is sufficient to produce an odor that will last for a day and possibly for a week. In conjunction with the test proper, Gadamer

advises preparing a second flask containing the materials used, that is, bread, water, calcium carbonate, and finally tartaric acid; and also a third flask like the first but containing in addition a small quantity (about 0.1 mg.) of arsenic trioxide. The second flask should not give a garlic odor, otherwise the materials contain arsenic. The third flask should give such an odor, otherwise the given material contains substances detrimental to the growth of the mould. In absence of selenium and tellurium, if the above conditions give rise to a garlic odor, presence of arsenic is proved; and its non-appearance means absence of arsenic. In point of delicacy this biological test is scarcely inferior to a chemical test for arsenic.

Notes.—Strong mineral acids, such as sulphuric and hydrochloric acid, prevent growth of the mould. This preventive action may be overcome by neutralization with calcium carbonate, which may be present in excess without ill effect. Alkalies also interfere with growth of the mould. They may be removed by neutralization with tartaric or citric acid, either of which may be present in excess. The great advantage of the biological over a purely chemical method lies in the fact that less time is required for a result. The tedious and unavoidable destruction of organic matter in material is unnecessary. Moreover, a number of tests for arsenic may be made at the same time.

Abel and Buttenberg (loc. cit.) say of this method: "The biological method of detecting arsenic has so many advantages, that it deserves to be recommended for the most varied purposes. Its application is very general and the method of procedure simple. The culture of mould may be kept a long time, even a year or more, without being revived. The test is very delicate and the odor readily recognized. Generation of the odor, in the case of cultures containing only 0.0001 gram of arsenic may be demonstrated for a week."

Besides being practically unlimited in application, the biological method is extraordinarily delicate. In this respect, it exceeds the best known chemical methods for detecting arsenic. It is, for example, considerably more delicate than Bettendorff's test, and possibly may equal the Marsh and Gutzeit tests in delicacy.

According to Scholtz,[1] the biological test for arsenic is even more delicate than the Marsh test. The latter fails in cases where the biological test gives the characteristic garlic odor very distinctly, for example, in the case of such quantities as 0,005–0,002 mg. of arsenic. To avoid error in testing urine on account of its odor, Scholtz advises boiling the urine beforehand with animal charcoal to remove this odor as much as possible.

After many experiments, Abba[2] was convinced of the delicacy, rapidity and reliability of Gosio's arsenic test. This test was especially useful in making over 100 tests of dried skins from the Indies frequently preserved with arsenical com-

[1] W. Scholtz: Biological Detection of Arsenic in Scales from Skin, Hair, Perspiration and Urine. Berl. klin. Wochenschr. 36, 913.

[2] F. Abba: The Delicacy of the Biological Method in Detecting Arsenic. Centralblatt f. Bakterien u. Parisitenkunde II. 4 (1898), 806.

pounds. The test for arsenic was made by placing in Petri dishes 2–3 slices of potato with holes punched in the middle. A piece of skin about 1 cm. long and 0.5 cm. wide was placed in each hole and then everything was sterilized for 20 minutes in an autoclave at 115°. The slices of potato were then inoculated with Penicillium brevicaule and kept at room temperature for 24 hours. The reaction took place in less than 24 hours at 37° but Abba preferred to carry out the test at room temperature.

Dry cultures of Penicillium brevicaule keep for a year, thus showing great resistance. On the other hand, growth is checked by acids and alkalies. The retarding influence of these substances almost always is easily overcome by proper treatment of material. Any free acid may be neutralized by calcium carbonate, or bases by tartaric acid and the latter neutralized by calcium carbonate. Phenols and mercury salts also retard growth of Penicillium brevicaule. It is advisable therefore first to make certain by special tests that Penicillium brevicaule will grow upon the material and also that it has the power of developing a garlic odor from arsenical compounds. It is also a fact that rather large quantities of arsenical substances prevent development of the mould.

Detection and Estimation of Arsenic

(Schneider[1], Fyfe[2], Beckurts[3])

The principle of this method is based upon the fact that trivalent as well as quinquevalent arsenic may be converted by hydrochloric acid into arsenic trichloride ($AsCl_3$) and the latter distilled in a current of this acid. In order that the process may proceed quantitatively, arsenic must be in the acid-soluble, trivalent condition and hydrochloric acid in such concentration that arsenic trichloride will pass over completely, since the reaction:

$$As_2O_3 + 6HCl \rightleftarrows 2AsCl_3 + 3H_2O$$

is reversible. Quinquevalent arsenic of arsenic acid passes over only in very small quantity, since the reaction:

$$H_3AsO_4 + 5HCl = 4H_2O + AsCl_5$$
$$AsCl_5 = AsCl_3 + Cl_2$$

does not run to completion. In presence of arsenic acid, Beckurts therefore recommends addition of ferrous chloride, whereby arsenious acid is formed:

$$H_3AsO_4 + 2HCl + 2FeCl_2 = H_3AsO_3 + H_2O + 2FeCl_3$$
$$H_3AsO_3 + 3HCl = AsCl_3 + 3H_2O$$

[1] Schneider: Wiener akadem. Berichte 6 (1850), 409 and Jahrbuch f. Chemie 1851, 630.

[2] Fyfe: Journ. f. prakt. Chemie 55, 103.

[3] H. Beckurts: Determination of Arsenic in Forensic-Chemical Examinations. Archiv d. Pharmazie 222 (1884), 653.

Schneider mixes the substance to be tested for arsenic with fused sodium chloride and evolves a stream of hydrogen chloride by adding concentrated arsenic-free sulphuric acid. Inasmuch as the formation of large quantities of salt causes trouble, and also unfits the distillation residue for many other examinations, it is advisable to pass into the material hydrogen chloride gas, that has been washed through arsenic-free concentrated hydrochloric acid, and distil arsenic trichloride in a stream of hydrogen chloride. This method is adapted especially for detection of arsenic in foods, beverages, various other materials, dyes, etc. When heated in a stream of hydrogen chloride, metallic arsenic, arsenic sulphide, and many organic compounds containing arsenic yield either no arsenic at all or only traces at most. In all such cases organic matter should first be destroyed by the Fresenius-von Babo method before distillation is undertaken.

Procedure.—Destroy organic matter in a weighed quantity of material by heating in the usual manner with concentrated hydrochloric acid and potassium chlorate. Then transfer the mixture to a capacious tubulated retort (Fig. 44). The neck of the latter should be directed obliquely upward and the end bent down at an obtuse angle (about 120°). This may be connected with a Liebig condenser, or better, as shown in the diagram, by means of an adapter with a liter flask serving as receiver. The latter should contain very little water and be well cooled in ice-water. The end of the adapter barely dips into the water. To prevent loss, the receiver is connected with a second, smaller flask also containing water. Add to the mixture in the retort 4–6 grams of ferrous chloride to reduce arsenic acid. Then pass through the tubulus of the retort a rapid stream of hydrogen chloride, generated by dropping concentrated arsenic-free sulphuric acid into fuming concentrated hydrochloric acid in a suction-flask. The use of a dropping-funnel permits regulation of the flow of sulphuric acid and consequently of evolution of hydrogen chloride. It usually requires 10–15 minutes to saturate the contents of the flask with hydrogen chloride. Then heat the retort and, while continuing the stream of hydrogen chloride, distil off about half the liquid. The distillate, containing arsenic exclusively in the trivalent condition, may be tested direct either by the Bettendorff or Gutzeit test. This distillate is said not to be adapted for examination in the Marsh apparatus, because hydrogen chloride carried over to some extent by hydrogen may prevent formation of

the arsenic mirror, or redissolve one already formed. A "zinc mirror" due to zinc chloride carried over may also appear. Opposed to these statements of Dragendorff and Otto, is the experience of Beckurts who always succeeded in detecting arsenic by the Marsh test in this distillate diluted with water, even when only traces were present. This distillate may also be used in testing for arsenic with Bougault's reagent.

The iodimetric method may be employed to determine arsenic quantitatively in this distillate. Since, however, in addition to trivalent arsenic, it may contain other substances derived from

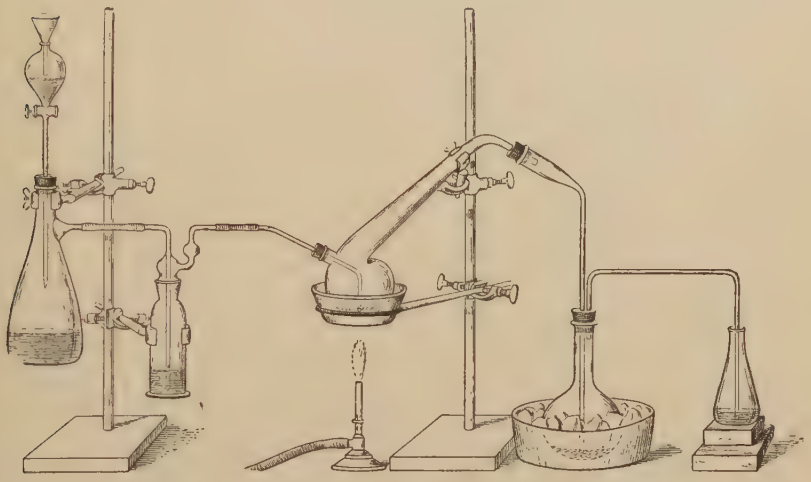


FIG. 44.—Distillation of arsenic as arsenic trichloride.

organic matter, such as furfural, which also consume iodine in alkaline bicarbonate solution, this distillate is not suitable for direct titration with 0.1 n-iodine solution. To remove these substances that interfere by combining with iodine, add saturated potassium permanganate to the distillate drop by drop, until the red color just persists. Then add 5–6 grams of ferrous chloride and resume distillation in the above apparatus in a stream of hydrogen chloride. Cool the distillate obtained and add small pieces of solid sodium hydroxide until the reaction is faintly alkaline. Again render faintly acid with dilute hydrochloric acid, add 5–10 grams of sodium bicarbonate, and titrate with 0.1 n-iodine solution to a blue color with starch solution as indicator.

Calculation.—The equation: $As_2O_3 + 2H_2O + 4I = As_2O_5 + 4HI$ is the basis of the calculation. 1000 cc. of 0.1 n-iodine solution correspond therefore to $As_2O_3/40$ grams = 4.95 grams of As_2O_3. 1 cc. of 0.1 n-iodine solution = 0.00495 gram of As_2O_3.

Notes.—The titre of the iodine solution depends more or less upon the degree of dilution. So the titre should be determined under the same conditions that prevail in the actual titration with 0.1 n-arsenious acid solution. If, for example, the volume of the liquid in which arsenic has been determined is 500 cc. and 10 cc. of 0.1 n-iodine solution were used, the titre is established by diluting 10 cc. of 0.1 n-arsenious acid solution to 500 cc. and titrating with 0.1 n-iodine solution after addition of solution of sodium bicarbonate and starch. The titre derived in this way is taken as the basis of the calculation.

Destruction of Organic Matter by Permanganate or Persulphate and Iodimetric Estimation of Arsenic

(Rupp and Lehmann[1])

The basis of this estimation of arsenic is the Schneider-Fyfe-Beckurts method, according to which arsenic is distilled as arsenic trichloride and titrated in the distillate with 0.01 n-iodine solution. Destruction of organic matter should be thorough, otherwise in addition to arsenic trichloride other substances that combine with iodine, such as furfural, may distil over. Rupp and Lehmann insure complete destruction by using potassium permanganate or persulphate in presence of sulphuric acid. Following destruction of organic matter, arsenic is distilled as trichloride in a stream of hydrogen chloride, evolved from the solution of the substance, and collected in a receiver containing plenty of sodium bicarbonate covered with water.

1. Treatment with Permanganate.—First triturate 20 grams of meat or organ in a porcelain dish with 10 grams of finely powdered potassium permanganate until as uniform as possible and then with 10 cc. of dilute sulphuric acid. Heat this mixture upon a boiling water-bath, at the same time stirring frequently. Then add to the nearly powdered mass, while still warm, 25 cc. of concentrated arsenic-free sulphuric acid in small portions with good stirring. The resultant liquid has a yellow to brownish color and contains no organic matter, or only very small particles.

2. Treatment with Persulphate.—Mix 20 grams of meat or organ with 10 grams of potassium persulphate. Pour 10 cc. of dilute sulphuric acid over this mixture, stir frequently for 10 minutes, and finally add 20 cc. of concentrated sulphuric acid. The action with persulphate is not as violent nor as energetic as with permanganate. A considerable part of the meat is not destroyed. For this reason the mixture should be heated to boiling upon an asbestos plate over a very low flame. As a result of this operation more of the meat particles will disappear, especially if crushed with a heavy glass rod. The resultant liquid, provided it has not been heated too strongly, should have a light yellow color.

There is little choice between these two methods of destroying organic matter. Rupp and Lehmann, however, give permanganate the preference because it is always available and more likely to be positively arsenic-free.

[1] E. Rupp and F. Lehmann: Quantitative Determination of Arsenic. Archiv. der Pharmazie 250 (1911), 382.

3. Distillation and Titration of Arsenic.—Manganese dioxide should be removed from the solution obtained by the permanganate treatment, to prevent evolution of chlorine by hydrogen chloride. For this purpose add about 30 cc. of 3 per cent. hydrogen peroxide solution. This quickly reacts with manganese dioxide with vigorous effervescence (O_2):

$$MnO_2 + H_2O_2 + H_2SO_4 = MnSO_4 + O_2 + 2H_2O$$

Any excess of hydrogen peroxide will be removed by the heat due to mixing with sulphuric acid. As soon as effervescence ceases, pour the liquid into a Kjeldahl flask, rinsing the dish with 30 cc. of concentrated sulphuric acid. Add 5 grams of anhydrous, or 10 grams of crystallized, ferrous sulphate, cool, and add 50 grams of sodium chloride. Distil from a sand-bath, using the ordinary Kjeldahl still-head. The latter is bent forward and connected by means of rubber with a 30–40 cm. glass tube, dipping into a suspension of 40 grams of sodium bicarbonate in 100 cc. of water in a capacious Erlenmeyer flask. As soon as all solid sodium bicarbonate in the flask has disappeared after shaking, distillation is finished and all arsenic has passed over into the distillate. Stop distillation by raising the stopper of the still-head, or by loosening the rubber connector, and rinse off the glass tube with a little water. Cool the distillate, make alkaline if necessary with sodium bicarbonate and filter. Then titrate, according to the quantity of arsenic suspected, with 0.1 or 0.01 n-iodine solution, using starch solution as indicator:

1 cc. 0.1 n-iodine solution = 0.00374 gram Arsenic,
1 cc. 0.01 n-iodine solution = 0.000374 gram Arsenic

Correction.—The current of hydrogen chloride probably always carries over into the distillate definite but small quantities of organic decomposition products that combine with iodine. For this reason Rupp and Lehmann made a series of blank tests, using the same quantities of the same kind of materials but arsenic-free. The quantity of iodine thus determined should be subtracted from the quantity of iodine required in the examination itself. Determinations made according to the procedure described showed that 20 grams of meat required 0.4–0.6, or on the average, 0.5 cc. of 0.01 n-iodine solution. A series of blank tests with smaller quantities of meat, that is, 10 or only 5 grams, again showed that the average consumption of iodine was 0.5 cc. of 0.01 n-iodine solution. This quantity should be subtracted from the volume required in the arsenic titration.

In the examination of fluid materials, such as blood and urine, Lehmann[1] advises the following procedure: Carefully evaporate 500 cc. of urine nearly to dryness with 2.5 grams of finely powdered potassium permanganate. If foaming becomes too troublesome, it may be prevented by adding 0.3–0.5 gram of paraffine. Then stir the residue with 5 grams of powdered potassium permanganate and 10 cc. of dilute sulphuric acid, running in after about 5 minutes 20 cc. of concentrated sulphuric acid. As soon as evolution of gas has ceased, expel chlorine by adding 30 cc. of 3 per cent. hydrogen peroxide solution and heating to boiling. Then transfer the mixture to a Kjeldahl flask, rinsing out the dish with 30 cc. of concentrated sulphuric acid and adding 10 grams of crystallized ferrous sulphate. Cool well, add 50 grams of sodium chloride, and distil as directed

[1] F. Lehmann: Quantitative Determination of Arsenic. Examination of Blood and Urine. Archiv. der Pharmazie 251 (1912), 1.

above. Shake thoroughly 50 grams of blood in a Kjeldahl flask with 2.5 grams of finely powdered potassium permanganate and after 10 minutes pour in 60 cc. of concentrated sulphuric acid in a thin stream. Cool and add in small portions 10 grams more of powdered potassium permanganate. Keep the mixture cool if it shows a tendency to warm up much. Shake frequently during 15 minutes, then add 30 cc. of hydrogen peroxide (3 per cent.) and 8 grams of anhydrous ferrous sulphate. Finally introduce 50 grams of sodium chloride, 5 grams of olive oil, and proceed with the distillation.

Iodine for Substances Not Arsenic.—As the mean of six blank tests with arsenic-free urine and blood, Lehmann found that 0.05 cc. of 0.1 or 0.5 cc. of 0.01 n-iodine solution was required. In an examination of urine or blood containing arsenic, this quantity should be subtracted from the volume found in titration.

Colorimetric Estimation of Minute Quantities of Arsenic in Parts of Cadavers (Führer[1])

In reality Führer's procedure is a composite of parts of other methods. He destroys organic matter by potassium permanganate as described by Rupp and Lehmann. He conducts the distillation according to Schneider and Fyfe, modifying the procedure of the former. Finally he estimates arsenic colorimetrically by the Gutzeit-Flückiger-Smith method, as lately described by Beck and Merres (see page 497), using the distillate containing hydrochloric acid direct for evolution of arsine. According to Führer, by combining these methods, the quantitative estimation of minute quantities of arsenic gives satisfactory results only when definite conditions as specified below are maintained.

1. Destruction of Cadaveric Material and Distillation

Triturate 10 grams of flesh, parts of organs, or other material in a porcelain mortar with 5 grams of finely powdered potassium permanganate. Then add 10 cc. of dilute sulphuric acid (1:5) and stir until uniform. Set the mortar upon a boiling water-bath, stirring frequently and keeping it there until its contents form a powdery mass. Usually this requires 20–25 minutes. Without cooling, run in slowly 25 cc. of concentrated sulphuric acid and then 6 per cent. hydrogen peroxide, obtained from freshly diluted perhydrol, until decolorization is complete. As a rule 15 cc. are sufficient. Now bring the volume of the residue to 30 cc. with water. As soon as foaming ceases, transfer the thick, yellow, opaque liquid to a Kjeldahl flask, rinsing out the mortar with 30 cc. of concentrated sulphuric acid. Heat the flask over a free flame until its contents are clear or, should this not take place, until boiling begins. The transparent, light brown liquid obtained again becomes turbid upon addition of 5 grams of crystallized ferrous sulphate. Cool thoroughly and add 50 grams of sodium chloride. At once close the flask (Fig. 45A) with a two-hole rubber stopper, through one opening of which passes a separating funnel (G) and through the other a Stutzer still-head (B). Connect the latter by means of a piece of black rubber tubing (F) (red rubber may give off antimony!) with a 50 cc. pipette (C), extending nearly to the bottom of the receiver (D). The receiver is a 150 cc. Erlenmeyer

[1] H. Führer: A Simple Procedure for Determining Minute Quantities of Arsenic in Parts of Cadavers. Berichte d. Deutsch. pharm. Ges. 28 (1918), 221.

flask provided with a doubly bored rubber stopper. Through one opening of the latter pass the pipette and through the other a calcium chloride tube (E) filled with glass beads. Pour into the receiver through the latter tube 50 cc. of 1 per cent. potassium iodide solution, a part of which is retained by the glass beads. Set the receiver in a vessel of cold water. Distillation requires about 45 minutes, when the Kjeldahl flask is heated in a sand-bath and the temperature is gradually raised. Provided the liquid is well cooled and fused salt is used, the flask contents do not froth enough to cause trouble. If ordinary powdered salt is used, addition of 10 drops of olive oil prescribed by Lehmann is helpful. Distillation is continued, until the bend (H) of the connecting tube is hot. Then all hydrogen chloride has passed over and the contents of the receiver begin to rise in

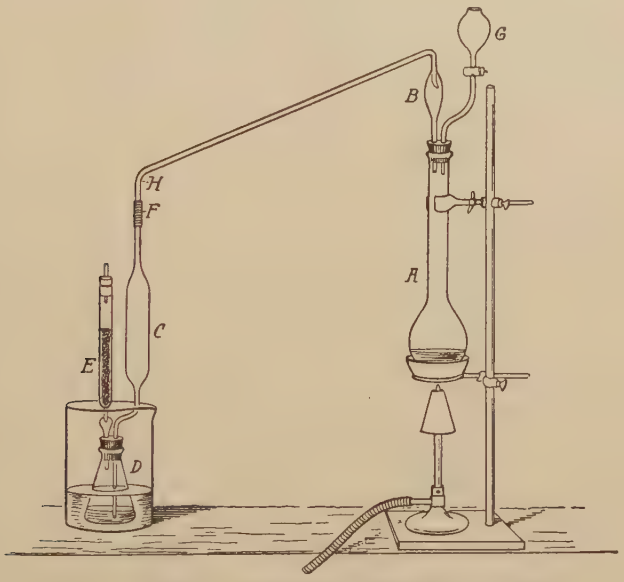


FIG. 45.—Fühner's apparatus for distillation of arsenic.

the pipette. Stop distillation by opening the stop-cock of the separatory funnel (G). The latter serves as a safety-tube and prevents the distillate from being suddenly sucked back. Remove the receiver together with pipette and cool. Rinse out the pipette and still-head carefully with water, transfer the light brown liquid to an Erlenmeyer flask, and bring the volume to 135 cc. Add 0.5 gram of stannous chloride. After a normal distillation this quantity is sufficient for complete decolorization. But if chlorine has been evolved during distillation from presence of undecomposed manganese dioxide or hydrogen peroxide, the potassium iodide solution becomes dark, or iodine may even separate out in the receiver and bulb-tube, so that several grams of stannous chloride may be required for complete reduction. This has a marked influence later upon evolution of hydrogen.

Notes.—In order that the distillate obtained by the above procedure, when treated with zinc, may give off arsine and produce colorations of the "mercuric bromide paper strips" that are comparable, concentration of hydrochloric acid in the receiver should always be the same. This is possible, if in each determination the same volume of liquid (H_2SO_4, H_2O_2) is used and the same weight of material is destroyed. Of even greater importance, however, is that the distillate be free from chlorine. To insure its absence, coloration due to manganese dioxide should be removed by hydrogen peroxide and the latter decomposed by boiling the liquid sufficiently long. Even traces of chlorine prevent evolution of arsine but possible interference on this account is obviated by receiving the arsenic trichloride in a solution of potassium iodide instead of in water. The color due to liberated iodine is discharged by stannous chloride. Although a little stannous chloride favors evolution of hydrogen, larger quantities exert a retarding action.

FIG. 46.—Führner apparatus for colorimetric estimation of arsenic.

2. Colorimetric Estimation of Arsenic in the Distillate

The distillate collected in the Erlenmeyer flask is colorless and turbid. After dilution to 135 cc. its hydrogen chloride concentration is about 17 per cent. In comparative experiments the temperature of the distillate should always be brought to 20°. Add 10 grams of zinc in the form of thin rods and at once close the flask with the tube attachment shown in Fig. 46. This consists of two Allihn-tubes, each 20 cm. long, and one glass tube of the same length having a bore of about 3 mm. Place in the first Allihn-tube strips of filter-paper moistened with lead acetate solution and in the second cotton threads saturated with the same solution. Hydrogen sulphide is absorbed by these tubes. The filter-paper always becomes colored and has to be frequently renewed, whereas the threads usually remain unchanged. Before the experiment, a strip of mercuric bromide paper is suspended in the third glass tube and so adjusted that its end is sufficiently low to be observed during the test. In order that the coloration may be the same on both sides, the lower end of the strip should hang exactly in the middle of the tube. The strip should not be removed until it has been exposed to the action for an hour.

Preparation of Mercuric Bromide Paper.—Cut strips of drawing paper 20 cm. long so that they may be exactly introduced into the tube. Place these for one hour in 5 per cent. alcoholic mercuric bromide solution. Then remove excess of solution by drawing the strips lightly between two fingers, protect from light, and dry between filter-paper. Preserve these strips in the dark in a stoppered glass tube. Thus protected they keep well and may be used for a long time.

Prepare the comparison-strips, required for the colorimetric estimation of arsenic, by means of solutions of pure arsenic trioxide in dilute sodium hydroxide

solution. Convenient dilutions for this purpose are the following: 1:1000 (1 cc. = 1 mg.); 1:10,000 (1 cc. = 0.1 mg.); 1:100,000 (1 cc. = 0.01 mg.); and 1:1,000,000 (1 cc. = 0.001 mg. As_2O_3). Since these extremely dilute arsenic solutions do not keep long, they should be freshly prepared for the comparison-strips. Such strips when prepared from these solutions should be pasted side by side and, if protected from light and air, they remain unchanged for a long time when kept in a book. When pure substances are used, Führer's method is applicable to the estimation of arsenic within the limits 0.005–0.5 mg. of As_2O_3.

Detection and Quantitative Estimation of Arsenic

(Engel and Bernard[1])

Hypophosphorous acid like Bettendorff's reagent completely reduces arsenic compounds in presence of concentrated hydrochloric acid to metallic arsenic:

$$2As_2O_3 + 3H_3PO_2 = As_4 + 3H_3PO_4$$
$$4AsCl_3 + 6H_2O + 3H_3PO_2 = As_4 + 12HCl + 3H_3PO_4$$

Moreover iodine solution oxidizes elementary arsenic to arsenic acid in a solution rendered alkaline by sodium bicarbonate.

$$2As + 5H_2O + 10\,I = As_2O_5 + 10HI$$
$$As_2O_5 + 3H_2O = 2H_3AsO_4$$

Procedure.—Precipitate arsenic from the hydrochloric acid solution of a weighed quantity of material with excess of hypophosphorous acid. Filter, wash thoroughly with water, and determine arsenic by titration with 0.1 n-iodine solution in presence of considerable sodium bicarbonate, using starch solution as indicator.

Calculation.—The equation above shows that 1 atomic weight of iodine corresponds to 0.2 atomic weight of arsenic = 15.0. So 1000 cc. of 0.1 n-iodine solution correspond to 1.5 gram of arsenic and 1 cc. = 0.0015 gram of arsenic.

Bougault[2] recommends the use of sodium hypophosphite (NaH_2PO_2) instead of free hypophosphorous acid, 20 grams of which are dissolved in 20 cc. of water with addition of 200 cc. of hydrochloric acid (sp. gr. 1.17 = 34.25 per cent.). Sodium chloride separates out and is filtered off by means of a cotton plug. The filtrate is Bougault's reagent for arsenic. This reagent has proved serviceable in detecting and determining arsenic in glycerol. The sensitiveness

[1] R. Engel and J. Bernard: A Rapid Method for the Quantitative Determination of Arsenic. Compt. rend. 122 (1896), 390 and Journ. Pharm. et Chimie (6) 3 (1896), 413.

[2] J. Bougault: Arsenic in Glycerol. Journ. Pharm. et Chim. (6) 15 (1902), 527.

of Bougault's reagent is considerably increased, if 1–2 drops of 0.1 n-iodine solution are added to the mixture of reagent and liquid being tested. Under these conditions a test in the cold will give a distinct coloration in 15 minutes even with 0.005 mg. of arsenic trioxide, and after 5 minutes a distinct cacodyl odor with 0.05 mg. of sodium cacodylate (Bougault).

Electrolytic Detection of Arsenic

To detect arsenic electrolytically, introduce the liquid, such as the sulphuric acid solution containing arsenic as arsenic acid obtained by the general procedure (see page 486), or urine or stomach con-

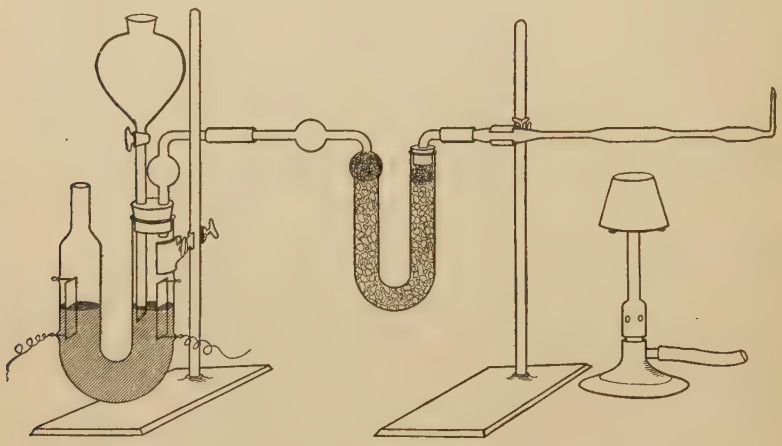


FIG. 47.—Apparatus for electrolytic detection of arsenic.

tents, into a sufficiently wide U-tube provided with platinum electrodes (Fig. 47). Pass the current through the liquid acidified with sulphuric acid. If it contains arsenic, arsine (AsH_3) together with hydrogen will appear at the cathode. Hydrogen should first be tested for arsenic by the Gutzeit test (see page 496). Arsenic is present, if a yellow spot appears on the paper moistened with saturated silver nitrate solution (1:1), Additional evidence of the presence of arsenic is afforded by connecting the U-tube, as shown in the sketch, with a calcium chloride tube and a Marsh reduction-tube. If the latter is heated to redness, an arsenic mirror will appear. The current used should have an electromotive force of 7–8 volts. The electrolytic method is especially serviceable in

detecting arsenic in inorganic compounds present in secretions, such as urine, but not arsenic in organic combination, such as cacodyl compounds and arrhenal. Among these organic compounds of arsenic, atoxyl, $H_2N.C_6H_4.AsO(ONa)_2$, is an exception. The arsenic being rather loosely bound is broken up by the electric current with formation of arsine.

Electrolytic Estimation of Minute Quantities of Arsenic

(Mai and Hurt[1])

By this method minute quantities of arsenic (fractions of a mg.) are separated quantitatively from an arsenical electrolyte at the cathode as arsine. The latter then reacts quantitatively with silver nitrate as follows:

$$AsH_3 + 3H_2O + 6AgNO_3 = H_3AsO_3 + 6HNO_3 + 6Ag$$

The advantages of the electrolytic detection of arsenic are, first avoidance of traces of arsenic that sometimes come from zinc in the Marsh test, and second the possibility in many instances of omitting destruction of organic matter. Thorpe[2] has shown the latter to be the case in the examination of beer-worts and malt-extracts for arsenic. To reduce arsenic acid and its salts, a few drops of zinc sulphate solution as electrolyte should be added to the sulphuric acid. The cathode is said to have a higher tension and the hydrogen to be very active.

Apparatus and Procedure.—The apparatus used by Mai and Hurt is shown in Fig. 48.

A is the reduction-tube and *B* a bulb-tube with 5–6 bulbs containing 0.01 n-silver nitrate solution. *A* and *B* are connected by a small tube *g* containing pieces of pumice-stone saturated with an alkaline lead solution, or glass-wool, to retain any traces of hydrogen sulphide. Anode *a* and cathode *e* are lead strips about 1–2 mm. thick. Their upper ends about 5 mm. thick are luted into glass tubes *b* which pass through the stopper of the U-tube and are tight. The dropping-funnel *d* holds about 25 cc. and its capillary end dips

[1] C. Mai and H. Hurt: Electrolytic Determination of Minute Quantities of Arsenic. Zeitschr. f. Unters. d. Nahrgs. u. Genussm. 9 (1903), 193.

[2] T. E. Thorpe: Electrolytic Determination of Minute Quantities of Arsenic, Especially in Brewery Materials. Proc. Chem. Soc. 19, 183; Journ. Chem. Soc. London 83 (1905), 974.

about 2 cm. into the solution to be electrolyzed. Tube *c* for escape of oxygen from the anode chamber contains a little water.

Fill U-tube *A* up to the mark with 12 per cent. arsenic-free sulphuric acid and bulb-tube *B* with 10 cc. of 0.01 n-silver nitrate solution. Turn on the current and keep at 2–3 amperes. If the silver nitrate solution remains unchanged after hydrogen has been running for 1 hour, the lead cathode and sulphuric acid are arsenic-free. Without stopping the current, introduce from the dropping-funnel *d* the solution to be tested for arsenic, the quantity of which

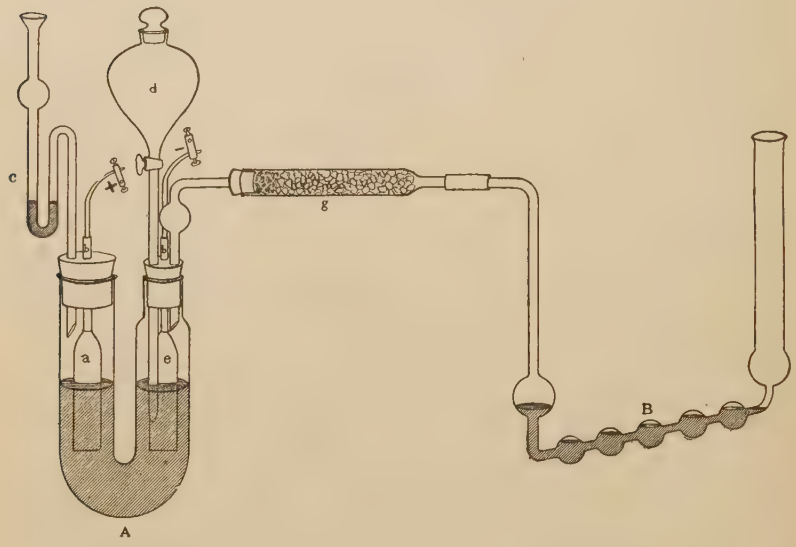


FIG. 48.—Mai and Hurt apparatus for electrolytic estimation of arsenic.

should not be more than 10 cc. Add this solution as slowly as possible and wash the last traces in with a little water. If the solution contains arsenic, or arsenic acid, in a few minutes the silver nitrate solution will become dark and the reaction will be at an end in 3 hours. Pour the contents of the bulb-tube through a small asbestos filter, wash with 3–4 cc. of water and titrate excess of 0.01 n-silver nitrate with 0.01 n-potassium sulphocyanate according to Volhard's method, using ferric alum solution as indicator.

Calculation and Notes.—The reaction above shows that 6 molecules of silver nitrate correspond to 1 atom of arsenic (=75). Therefore 1 gram-molecule of silver nitrate = $\frac{1}{6}$ gram-atom of arsenic = $\frac{75}{6}$ = 12.5 grams of arsenic, and 1000

cc. of 0.01 n-silver nitrate = 0.125 gram of arsenic, or 0.1655 gram of As_2O_3, or 0.1916 gram of As_2O_5.

Electrodes of platinum (foil or gauze) can not be used in the electrolytic separation of arsenic as arsine, because either solid arsine or elementary arsenic is formed. Mai and Hurt also found that gold, silver and tin cathodes gave unsatisfactory results and carbon electrodes were not much better. Pure lead alone meets all the requirements as a material for the electrodes. Oxygen compounds of arsenic are quickly and completely reduced to gaseous arsine only upon cathodes of absolutely pure lead. The attachment of a platinum wire to a lead electrode was sufficient to cause incomplete reduction of arsenic compounds. For this reason the electrodes consist of one piece of lead[1] without soldering on wire of another metal. The best electrolyte is 12 per cent. sulphuric acid. A stronger acid easily causes formation of hydrogen sulphide and a weaker acid has the disadvantage of lower conductivity and lower specific gravity. The electrolyte should be specifically heavier than the solution to be tested, to keep the latter from passing at once to the bottom of the reduction-tube.

Mai and Hurt found the following quantities of arsenic:

	As taken	As found
As_2O_3	0.25 mg.	0.223 mg.
As_2O_3	0.10 mg.	0.099 mg.
As_2O_5	0.10 mg.	0.105 mg.

For qualitative tests the bulb-tube may be replaced by the drying and ignition-tubes of the Marsh apparatus.

According to Mai and Hurt, the statements of Thorpe and Trotmann, that every solution may be electrolyzed without previously destroying organic matter, do not always hold. In the examination of beer containing arsenic the results were fairly satisfactory, but in the case of urine the results were far too high.

Examination of Parts of the Cadaver and Other Organic Material

The electrolytic method, according to Mai,[2] is suitable for the detection and quantitative estimation of arsenic in parts of the cadaver. Complete destruction of organic matter is unnecessary, for arsenic is easily and completely separated, if destruction of organic matter is carried to the point where hard carbon is formed and distillation with hydrogen chloride is then undertaken. Finely comminuted material should be freed from liquid as completely as possible by drying in a porcelain dish upon a water-bath. If necessary, it should again be finely divided and brought to complete liquefaction in the same dish by warming with an equal weight of fuming nitric acid containing 5 per cent. of sulphuric acid. This is

[1] Kahlbaum's purest lead.

[2] C. Mai: Quantitative Determination of Arsenic for Forensic-chemical Purposes. Zeitschr. f. unters. d. Nahrgs. u. Genussm. 10 (1905), 242.

accomplished relatively quickly. Transfer the resultant liquid at once or in portions, depending upon its volume, to a smaller porcelain dish and heat first upon a sand-bath and finally over a free flame until excess of acid has been expelled and a hard glassy carbonaceous residue is left. Remove the latter from the dish and reduce to powder. Introduce this product into a round-bottom Jena flask with 5–6 times its weight of concentrated hydrochloric acid (sp. gr. 1.19 = 37.9 per cent.). Distil over a free flame to a third or a fourth of the volume, using a good condenser. All arsenic will distil over as arsenic trichloride. A reducing agent in this distillation is unnecessary, since arsenic acid is readily converted to arsenic trichloride by carbon and hydrochloric acid. Transfer the distillate to a smaller porcelain dish and evaporate upon the water-bath almost to dryness. While this is going on, run in gradually a third of its volume of fuming nitric acid. Finally heat the residue with a little concentrated sulphuric acid until abundant white fumes are given off. Bring the contents of the dish to proper dilution with water and transfer without loss to the dropping-funnel of the apparatus described elsewhere (see page 520). In other respects the determination is conducted in the manner already described.

Notes.—As compared with other methods, the principal advantage possibly of this procedure, aside from its simplicity, consists in limiting to three the large number of reagents usually required. Cane-sugar, invert-sugar, acetic acid, oxalic acid and tartaric acid, which may be in the cathode-chamber, have no influence whatever upon this arsenic determination. Equally insignificant is the effect of small quantities of hydrochloric acid. But a large quantity of this acid in the cathode-chamber should be avoided. All oxidizing agents, that is, nitric acid, must without exception be excluded, since traces appreciably diminish the quantity of arsenic obtained.

Procedure with Frerichs and Rodenberg's[1] Apparatus

Frerichs and Rodenberg made examinations by Mai and Hurt's method and confirmed its usefulness. They found the absorption-tube especially practical. But the long time required for complete reduction of arsenious to arsenic acid was a disadvantage. On this account they made the following change in the apparatus. They used a two-cell apparatus somewhat like that of Trotmann, but substituted for his parchment diaphragm, which is not durable, one of earthen ware and used lead for electrodes instead of platinum.

[1] H. Frerichs and G. Rodenberg: Electrolytic Determination of Small Quantities of Arsenic. Archiv d. Pharmazie 243 (1905), 348.

By means of caoutchouc, they luted a glass tube (b) 20 cm. long into an earthen cell (c) 3–4 cm. internal diameter and cut off at a height of about 5 cm. (Fig. 49). This glass tube is closed by a rubber stopper through which pass a dropping-funnel, the cathode (a) and a glass exit-tube. As in Mai and Hurt's apparatus, the mixture

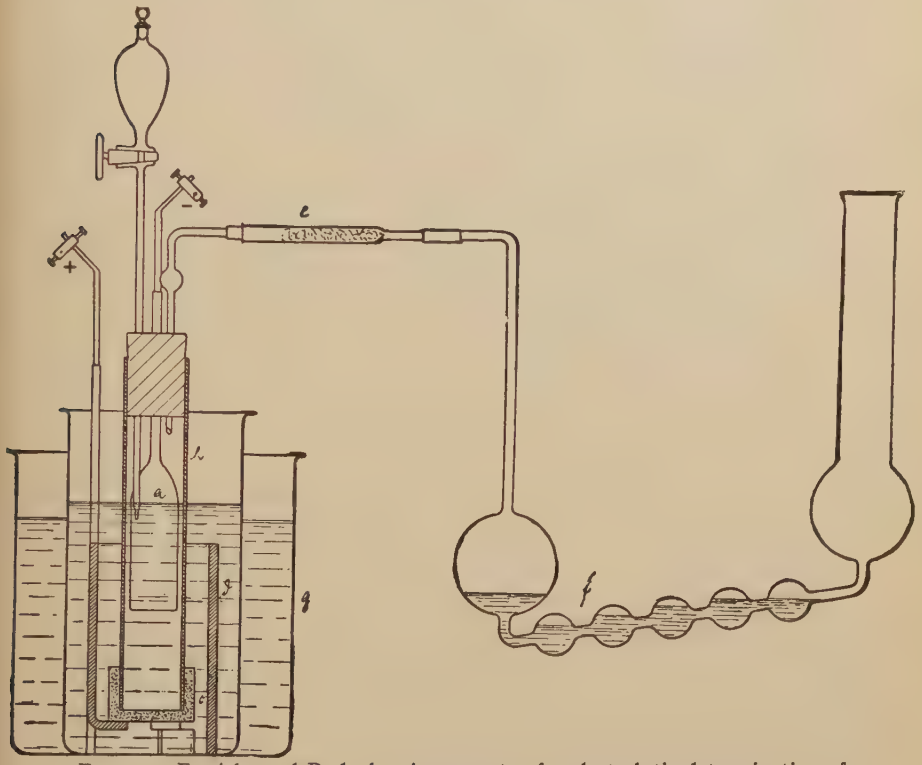


FIG. 49.—Frerichs and Rodenberg's apparatus for electrolytic determination of arsenic.

of hydrogen and arsine passes through a glass tube, containing pieces of pumice-stone saturated with basic lead acetate to hold back hydrogen sulphide, and then into the Mai-Hurt absorption-tube (f). Serving as anode is the lead plate (d). This does not entirely surround cylinder (b) and by means of a piece of about 1 cm. long cut out and bent inward supports the earthen cell so that the latter does not rest on the bottom of beaker (e). The entire decomposition-vessel (e) stands in a larger beaker (g) filled with water.

A current of 2–3 amperes having a tension of 16 volts will quantitatively convert 20 mg. of As_2O_3 in 30 minutes into AsH_3. If arsenic acid (H_3AsO_4) is present, that is, quinquevalent arsenic, it should be reduced by sulphurous acid but any excess of the latter must be expelled by warming upon the water-bath. Without this preliminary reduction, at least 3 hours are required to reduce quantitatively 15 mg. of As_2O_5 to AsH_3. The apparatus of Mai and Hurt, which has no diaphragm, takes even longer. The solution to be electrolyzed in the Frerichs-Rodenberg apparatus should have a sulphuric acid concentration of about 12 per cent.

In order to determine electrolytically the quantity of arsenic in any kind of cadaveric material, it should be prepared as described on page 521. Urine should not be electrolyzed direct, otherwise reduction of silver nitrate solution may occur even in absence of arsenic. Urine should be evaporated with fuming nitric acid and the residue heated with arsenic-free concentrated sulphuric acid until abundant white fumes are given off. The residue may then be diluted with water (10 cc.) and brought into the cathode-chamber to be electrolyzed.

Colorimetric Estimation of Small Quantities of Arsenic

(Autenrieth and Breh[1])

This method is based upon the fact that arsenic acid and salts are completely precipitated by uranyl acetate as uranyl arsenate, $(UO_2)HAsO_4.4H_2O$, or as uranyl ammonium arsenate, $(UO_2)(NH_4)AsO_4$, in presence of ammonium salts:

$$Na_2HAsO_4 + (C_2H_3O_2)_2UO_2 = (UO_2)HAsO_4 + 2C_2H_3O_2Na$$

$$Na_2HAsO_4 + (NH_4)Cl + (C_2H_3O_2)_2UO_2 = (UO_2)(NH_4)AsO_4 + NaCl + C_2H_3O_2Na + C_2H_4O_2$$

Both precipitates are practically insoluble in water. So they may easily be freed from soluble uranium salts by washing with water. But they are readily soluble in dilute nitric acid. In such a dilute nitric acid solution uranium may then be determined colorimetrically by means of the potassium ferrocyanide reaction. In very dilute solutions uranyl ferrocyanide forms and remains in colloidal solution, since the red-brown solutions that appear are entirely clear and their color strength may easily be determined colorimetrically. Flocks of uranyl ferrocyanide, $(UO_2)_2Fe(CN)_6$, first begin to appear at a concentration of 4–5 mg. of arsenic in 100 cc. of solution. By this method is determined not the arsenic but the uranium present in the precipitate. But since in uranyl arsenate and uranyl ammonium arsenate a uranyl radical is present for every atom of quinquevalent arsenic, these indirect determinations give correct arsenic values. Trivalent arsenic must first be converted to arsenic acid by some oxidizing agent, such as bromine water, fuming nitric acid, or potassium chlorate and hydrochloric acid. The very convenient mixture of ammonia and hydrogen peroxide cannot be used,

[1] As yet unpublished by the authors.

since the latter often contains phosphoric acid. Sometimes this acid is used in the manufacture of hydrogen peroxide, or it may have been added to increase its keeping properties. Should the material contain phosphoric acid, arsenic must first be precipitated in hydrochloric acid solution by hydrogen sulphide, otherwise uranyl phosphate, $(UO_2)HPO_4$, will be mixed with uranyl arsenate.

Procedure.—Render the aqueous solution of arsenic acid, or arsenate, which should be free from phosphoric acid, strongly alkaline with ammonia, and then faintly acid with acetic acid. Add uranyl acetate solution in excess and heat to boiling. Allow to settle for 15–30 minutes and filter the precipitate through a rather small paper. Usually it is necessary to pour the filtrate repeatedly through the paper until it comes absolutely clear. Wash the precipitate with warm water containing a few drops of ammonium chloride solution, until the cool filtrate with 2–3 drops of dilute nitric acid gives no color with potassium ferrocyanide. Unless an electrolyte is added to the wash-water, uranyl arsenate will partly go into colloidal solution. Dissolve the washed precipitate upon the filter in a few drops of dilute nitric acid, thoroughly washing it out with warm water. Here again the filtrate must be repeatedly passed through the paper to dissolve the last traces of precipitate. Collect filtrate and wash-water in a graduate, or graduated flask, holding 50–100 cc., add 2–3 cc. of 10 per cent. potassium ferrocyanide solution, shake and allow to stand for 10–20 minutes until the development of color has reached a maximum. Dilute, if necessary, with water to a definite volume so that the color is of such depth that it may be conveniently compared with the standard. For colorimetric examination such a solution should be perfectly clear and have a pure red-brown color. Solutions containing 3–3.5 mg. of arsenic in 100 cc. remain perfectly clear after standing for some time. Should flocks of uranyl ferrocyanide appear, a colorimetric determination is no longer possible. To make such a determination, fill the trough of the Autenrieth-Koenigsberger wedge-colorimeter (see page 468) with the color solution, adjust the calibrated comparison-wedge so that the intensity of color is equal to that of the solution examined. Read on the scale of the colorimeter the point at which this occurs. The calibration-curve, or calibration-table, will give in mg. the arsenic value corresponding to the observed reading.

Calibration of the Comparison-wedge

The comparison-wedge can not possibly be filled with a clear solution of uranyl ferrocyanide because after a time flocks of this substance begin to appear. For this reason a permanent artificial solution is prepared by properly mixing aqueous solutions of cobaltous nitrate, chrome alum, copper sulphate and picric acid. The glass-stoppered wedge is filled with such a solution. To calibrate the wedge, an arsenic acid solution containing 1 mg. of arsenic per cc. is prepared. Since 1 mg. of arsenic is contained in 0.266 cc. of 0.1 n-arsenious acid solution, measure accurately 26.6 cc. of the latter, evaporate repeatedly upon the water-bath with bromine water, and bring the volume of the residue to 100 cc. with water. 1 cc. of this solution contains 1 mg. of arsenic. In the manner described above, precipitate arsenic acid from 3 cc. of this solution (= 3 mg. of arsenic) and prepare with the washed precipitate 100 cc. of color solution. The more dilute color solutions were prepared by diluting this solution with water. Then the color

strength of each of these solutions was determined in the colorimeter by means of the comparison-wedge:

Color solution in cc	10	9	8	6	5	4	2
Water in cc	0	1	2	4	5	6	8
As in mg. in 1 cc.	0.03	0.027	0.024	0.018	0.015	0.012	0.006
Scale reading	43	49	55	67	73	79	92

If the arsenic values in mg. are recorded on coordinate paper on the abscissae and scale-readings for equal color strength on the ordinates, the calibration-curve of the comparison-wedge is obtained by connecting the points (see instructions, page 346). For the purpose of checking the correctness of this curve, a few points were verified with definite quantities of arsenic:

Theoretical value	0.1	0.5	1.0	2.1	3.0 mg.
Value obtained	0.09	0.6	0.95	2.0	2.8 mg.

Estimation of Arsenic in Urine and Organs

By prolonged heating with hydrochloric acid and potassium chlorate, destroy as completely as possible in the usual manner organic matter in urine and organs. After warming the filtrate for 0.5–1 hour and cooling, pass in arsenic-free hydrogen sulphide. After the precipitate has stood for some time, preferably until the next day, filter and wash carefully with dilute hydrogen sulphide water to remove completely any phosphoric acid that may be present. Repeatedly extract the precipitate on the filter with hot ammonia and evaporate the filtrate. Since the residue still contains organic matter, it must be freed from this by evaporation with a little hydrochloric acid and potassium chlorate. Usually the aqueous solution of the residue treated in this manner is pure white. Acidify faintly with acetic acid and precipitate with uranyl acetate as directed above. The following experimental results confirm the accuracy of this colorimetric method:

(a) **Experiments with Urine.**—In each instance 50 cc. of urine, containing varying quantities of arsenic, gave the following results:

Theoretical value	1.0	1.0	2.0	2.1	2.5	3.0 mg. of As
Value obtained	0.9	0.8	1.5	1.7	1.6	2.8 mg. of As

(b) **Experiments with Liver.**—In each instance 50 grams of liver, mixed with varying quantities of arsenic, gave the following results:

Theoretical value	2.2	1.5	1.5 mg. of As
Value obtained	1.8	0.68	0.85 mg. of As

LEAD

Detection of Small Quantities of Lead in Urine

In lead poisoning the quantity of lead in urine is usually very small and seldom amounts to more than a few mg. during 24 hours. Schumm[1] recently

[1] O. Schumm: Detection of Small Quantities of Lead in Urine. Zeitschr. f. physiolog. Chemie 118 (1922), 189.

tested the accuracy and reliability of various methods of detecting minute quantities of lead in urine and found the one recommended by Meillère[1] well adapted for this purpose. In this method lead-free copper sulphate is added to the solution to be tested and both metals are precipitated by hydrogen sulphide. Add 0.25–0.5 gram of crystallized copper sulphate and 5–10 cc. of 25 per cent. hydrochloric acid to the given liquid, pass in hydrogen sulphide first in the cold and then after warming, and allow to stand for 24 hours. Filter through ash-free paper and wash the precipitate twice with warm water. While still moist, boil out precipitate and filter with 10–14 per cent. nitric acid and wash! The filtered extract, according to Schumm, should not be evaporated to dryness and ignited as recommended by Meillère but only concentrated, then diluted with water until the volume is about that of the nitric acid used in extracting the filter, and electrolyzed. A convenient current is one of about 4 volts with two lead accumulators connected in series. By this method very small quantities of lead mixed with copper sulphide may be separated from a large quantity of urine. When electrolysis is completed, wash the electrodes with water, pour pure nitric acid over them, and add sodium nitrite which easily dissolves lead peroxide on the anode. Then heat the acid to boiling, wash the electrodes, pour off the liquid and evaporate upon the water-bath. Dissolve the residue in a few drops of water and treat the solution with hydrogen sulphide. When more than traces of lead are present, lead sulphide will separate in a flocculent condition. Filter and dissolve the precipitate on the filter with a little hot nitric acid. Evaporate this solution, take up the residue in a few drops of water, render alkaline with a trace of sodium hydroxide solution, add a drop of potassium chromate solution, and acidify with acetic acid. When the quantity of lead is very minute or doubtful, as may be the case if hydrogen sulphide produces no filterable flocks of lead sulphide, add a trace of sodium hydroxide solution, then nitric acid in excess, and evaporate the mixture. Dissolve the residue in a little water and test for lead with potassium chromate as directed above.

Experiments with Protein-free Human Urine

(Schumm)

1. Urine (700 cc.), containing 1 mg. of lead nitrate and 0.3 gram of $CuSO_4 \cdot 5\,H_2O$, was treated with 5 cc. of 25 per cent. hydrochloric acid. At intervals of 15 minutes this solution was then treated with hydrogen sulphide, first at room temperature and finally at about 70–80°. The precipitate was collected on a filter, washed with water, and carefully extracted with 10 cc. of boiling 25 per cent. nitric acid by repeatedly returning the filtrate to the paper and finally washing with water. The total filtrate was evaporated somewhat and electrolyzed with a small, clean platinum dish as cathode and as anode a movable platinum electrode having an active surface of about 3 sq. cm. and dulled or etched by aqua regia. The tension was 4 volts and the strength of current in the beginning 0.4–0.5 amperes. The time of electrolysis was 5 hours. The anode was brown in spots which immediately dissolved in the mixture of nitric acid and sodium nitrite. The extract treated in the manner described above gave good tests for lead.

[1] G. Meillère: Annal. de Chim. analyt. appl. 20 (1916), 73.

2. Urine (1500 cc.) was mixed with 0.2 mg. of $Pb(NO_3)_2$, 0.5 gram of $CuSO_4 \cdot 5\,H_2O$, and 10 cc. of 25 per cent. hydrochloric acid. Hydrogen sulphide treatment was the same as in Exp. 1, except that the temperature was raised only to about 50°. Electrolysis was carried on for 18 hours. The extract of the anode gave distinct tests for lead both with hydrogen sulphide and potassium chromate.

Notes.—Without further treatment the nitric acid solution of the hydrogen sulphide precipitate may be electrolyzed after concentration and dilution with water. Continuation of electrolysis until appearance of an alkaline reaction does not prevent separation of lead. The extract of the hydrogen sulphide precipitate with nitric acid is evaporated to dryness, the residue ignited and then dissolved in warm dilute nitric acid. The electrolytic separation of lead, especially when little acid is used, then takes place in 1–2 hours. Copper separates as a fine red deposit on the cathode and lead on the anode as a uniform, light brown coating. The omission of addition of copper sulphate, as recommended by Meillère, to the urine and treatment of the latter in presence of little acid with hydrogen sulphide makes it impossible to find such small quantities as 0.1 mg. of lead. Presence of albumin in urine may also interfere with the process as described.

MERCURY

I. Qualtitative Detection of Mercury in Urine

(Bardach[1])

Even after treatment with mercury of rather long duration, the daily human urine always contains very minute quantities of mercury, frequently only fractions of a mg. For this reason the recommendation has been made that the metal be concentrated before its direct separation by means of a precipitate that shall contain all the mercury in the urine. Stuckowenkow and Malkes[2] have devised a quantitative method based upon such a precipitation. In their method they have made use of the property possessed by protein substances of precipitating mercury and of the solubility of such precipitates of mercury-albuminate in hydrochloric acid. Mercury in this manner is concentrated in a small volume of liquid from which it may be separated by means of copper.

Procedure.—Pour into a flask with not too narrow a neck 500 cc. or more of unfiltered urine[3] to be tested for mercury and then add in small quantities and with good shaking 1 gram of commercially pure egg albumin very finely powdered. Allow the mixture to stand for several minutes to insure complete solution. Then render the urine acid with acetic acid. Normal urine (500 cc.) usually requires 5–7 cc. of 30 per cent. acetic acid. Suspend the flask in a hot water-bath, the water of which is kept actively boiling for about 15 minutes. Filter hot through a rather small paper. Drain the precipitate thoroughly and spread out the paper upon two thicknesses of filter-paper. Then transfer paper and precipitate to a small Erlenmeyer flask and pour in 10 cc. of concentrated

[1] B. Bardach: Detection of Mercury in Urine. Chem. Ztg. 24 (1901), 816.

[2] Stuckowenkow and J. Malkes: Quantitative Estimation of Mercury in Urine. Chem. Ztg. 24 (1901), 816.

[3] In urine containing albumin all or nearly all the mercury is in the sediment as albuminate.

hydrochloric acid (sp. gr. 1.19 = 37.9 per cent.). Shake well and introduce a bright copper spiral 2 cm. long, made from a piece of fine wire 40 cm. long, or a wire gauze. Cover the flask with a funnel, or watch-glass, and suspend it for 45 minutes in a boiling water-bath. Cool, pour off the acid, wash the spiral three times with water, then with alcohol and ether, and finally dry upon filter-paper. Insert the dry spiral into a glass tube, 14 cm. long and sealed at one end, of such bore that the spiral fits easily without rubbing. Drop in a very small particle of iodine and while turning the tube gradually heat in a small flame to faint redness. At once at the point where heat is applied a yellow to reddish ring of mercuric iodide will appear if the urine contained mercury. This "ring" may be sublimed higher with heat and becomes distinctly visible, if the tube is held against a black plate or paper. According to Bardach, the limit of delicacy of this qualitative test lies between 0.025–0.05 mg. of mercury. Precipitated uric acid, urates, oxalates, phosphates, moderate quantities of sugar, pathological albumin, and form-elements are without influence upon this test for mercury in urine.

Method of F. Glaser and A. Isenburg[1]

To precipitate mercury from urine, Glaser and Isenburg employ freshly precipitated aluminum hydroxide upon the principle that amorphous, voluminous precipitates separate mercury from its solutions through adsorption. Add 5 grams of aluminum sulphate (Aluminum sulphuricum purissimum cryst. Merck) to 250 cc. of well-shaken, unfiltered urine. Precipitate boiling with ammonia, filter hot by suction, wash with hot water, and dissolve the precipitate in concentrated hydrochloric acid. Introduce a copper spiral into this solution and set upon a boiling water-bath for 45 minutes. Remove the amalgamated copper spiral, wash according to the method of Bardach, dry and test for mercury as iodide. Other amorphous, voluminous precipitates are probably equally well suited for separating mecury from urine.

Method of Salkowski[2]

Salkowski has shown that urine containing mercury may be evaporated without loss of metal by acidifying with hydrochloric acid and avoiding evaporation to complete dryness. Add 2 cc. of hydrochloric acid (25 per cent. HCl) to each 500 cc. of urine and evaporate until salts begin to separate. Then oxidize organic matter with hydrochloric acid and potassium chlorate. Next extract with alcohol in which mercuric iodide is soluble, filter this alcoholic extract and evaporate. Take up the residue with 40 cc. of alcohol and add 60 cc. of ether. Allow this alcohol-ether extract to stand for a short time, filter, evaporate, and take up the residue in 10 cc. of water, filtering after thorough shaking. Introduce into this solution bright copper strips, leaving them there for about 1.5 hours. Wash these metal strips with water, alcohol and ether, dry, and heat in a dry test-tube. When

[1] F. Glaser and A. Isenburg: Detection of Mercury in Urine. Chem. Ztg. 33 (1909), 1258.

[2] E. Salkowski: Detection of Mercury in Urine. I and II. Zeitschr. f. physiolog. Chem. 72 (1911), 387; 73 (1911), 401.

cold, remove these strips, introduce a trace of iodine, warm gently, and remove excess of iodine by blowing in air. Then set the test-tube aside for several hours until the deposit of mercuric iodide forms. According to this procedure, a urine, containing 0.25 mg. of $HgCl_2$ in 500 cc., will give a distinct test for mercury.

In his second investigation, Salkowski recommends a somewhat different procedure whereby he avoids interference with the detection of mercuric iodide due to separation of iodine. Introduce a minute particle of iodine into the test-tube, placing the latter in a flat-bottom flask about one-third filled with water so that the tops of flask and test-tube are on a level. Keep in this position by means of filter-paper, or by winding wire around, but so that steam may pass by freely. Boil the water until the test-tube is as full as possible of iodine vapor. As soon as the red or yellowish deposit of mercuric iodide has formed, take out the test-tube and at once expel excess of iodine by an air-current from a bellows. If no mercuric iodide can be detected during heating, set the test-tube aside for some time. Then warm again and expel excess of iodine by air as above.

Modification by Oppenheim[1] of Jolles[2] Method

Oppenheim has adopted the following procedure in carrying out the method described by Jolles for the qualitative detection of mercury in urine. Warm 150–200 cc. of urine in a beaker with 5–10 cc. of chlorine-free concentrated hydrochloric acid and add 1–2 grams of potassium chlorate. Addition of potassium chlorate may be omitted, if the urine contains no albumin, and the test completed in less time. Add distilled water and boil the liquid gently until free chlorine can not be detected. The cooled liquid should not give a violet color with zinc iodide-starch solution. Now lower into the liquid a galvanic, gold-plated platinum foil (corrugated electrodes about 8 cm. long and 8 cm. wide made by C. W. Heraeus in Hanau of gold-plated platinum are most convenient for this purpose). Add 2–3 grams of crystallized stannous chloride and sufficient concentrated hydrochloric acid, about 30–40 cc., to render the liquid clear. Keep at a gentle boil for 15 minutes. Remove the platinum plate, rinse with distilled water, and place in a dish with a flat bottom. Add enough dilute nitric acid (1:4) to cover the plate and warm upon the water-bath until the volume of the nitric acid solution is reduced to 4 cc. Cool this solution in a test-tube and add about 4 cc. of freshly prepared hydrogen sulphide water. Make this test in duplicate with the control-solution (see below) obtained in the beginning by boiling out the platinum plate. Compare both colorimetrically. Mercury, if present, will appear in the first test-tube as a yellow to yellow-brown color, depending upon the quantity of mercury present.

Control Liquid.—The corrugated, gold-plated platinum electrode before each test should be warmed for 15 minutes in a flat dish upon the water-bath with dilute nitric acid (1:4) The solution obtained should be evaporated to about 4 cc. and used as a check upon the test proper.

[1] M. Oppenheim: Detection of Mercury in Urine. Zeitschr. f. analyt. Chem. 42 (1903), 431.

[2] A. Jolles: A Rapid and Exact Method of Detecting Mercury in Urine. Monatsh. f. Chem. 21 (1900), 352.

Method of Perelstein and Abelin[1]

Add 10 cc. of concentrated hydrochloric acid to 500 cc. or more of urine, boil for 1 minute and quickly cool. Add 5–6 cc. of ammonia, or a few cc. of sodium hydroxide solution, leaving the reaction faintly acid, then 20–25 grams of crystallized sodium acetate and 10 cc. of 10 per cent. ferric chloride solution. Again heat to boiling and filter hot, washing with hot water the precipitate which contains all the mercury in the urine. Dissolve the moist precipitate, best in a mortar, with as little concentrated hydrochloric acid as possible. Transfer this final solution of 10–15 cc. to a crystallizing dish and introduce a strip of bright sheet copper 4–5 mm. wide and about 15 mm. long. After 2 hours pour off the solution, wash the strip with water, alcohol and ether, dry in air, and heat carefully in a test-tube. After removing the strip, add a minute particle of iodine, and apply gentle heat. A reddish or yellowish deposit of mercuric iodide will appear, when only 0.1 mg. of mercury is present.

II. Quantitative Estimation of Mercury in Urine

(Schuhmacher and Jung[2])

In this method the mercurial urine is treated with hydrochloric acid and potassium chlorate, mercury precipitated with stannous chloride, and the precipitate collected in a weighed tube of potash glass containing gold-asbestos and weighed.

Procedure.—Add 100 cc. of concentrated hydrochloric acid and 15 grams of potassium chlorate to 1000 cc. of urine and bring at once to a boil in a 2000 cc. flask of potash glass over a wire-gauze. Then cool in running water and, when the temperature is 40–50°, add 50 cc. of clear stannous chloride solution prepared by dissolving pure tin in hydrochloric acid. Test a small portion of the clear solution with mercuric chloride solution for excess of stannous chloride. This control-solution should show reduction to grayish black mercury. If stannous chloride is present in excess, after 5 minutes standing filter by suction through a funnel provided with a tight asbestos filter. Wash with a little water and transfer asbestos and mercury, which still contain some organic matter, to a 250 cc. flask, rinsing out the funnel and adding a little sodium hydroxide solution. Allow the sodium hydroxide to act for a few minutes, then add a few grams of potassium chlorate, acidify strongly with hydrochloric acid, and warm over a wire-gauze until chlorine is completely expelled. This treatment destroys the last trace of organic matter. A condenser is unnecessary in this operation. Filter by suction into a thick-wall, round-bottom flask and add 10–20 cc. of clear stannous chloride solution to the warm filtrate. Filter the precipitated mercury, now free from organic matter, by suction through a tube of potash glass containing gold-asbestos,[3] so-called filter amalgam-tube (Fig. 50). Wash first with dilute

[1] M. Perelstein and J. Abelin: A Delicate Clinical Method of Detecting Mercury in Urine. Münch. Med. Wochenschr. 62 (1915), 1181.

[2] Schuhmacher and W. Jung: A Clinical Method of Estimating Mercury in Urine. Arch f. exper. Path. u. Pharm. 62, 138; Zeitschr. f. analyt. Chem. 39 (1900), 12; Ibid. 41 (1902), 461.

[3] In their second paper Schuhmacher and Jung state that granular gold is not absolutely necessary.

hydrochloric acid, then with water, and three times each with alcohol and ether. Dry thoroughly in a current of dry air, warming very gently. Weigh the tube, ignite strongly in a current of dry air, and again weigh when cold (Fig. 51). Obviously the mercury expelled by ignition may be condensed beyond the tube and tested qualitatively. The difference between the two weighings represents the quantity of mercury that was in the urine.

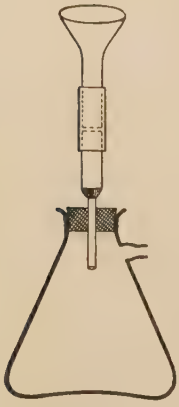


FIG. 50.

Gold-asbestos.—The material essential to this method is prepared by dissolving chemically pure gold in aqua regia, evaporating until very little acid remains, and introducing purified fine asbestos fibers into the solution. Drain the asbestos when saturated with the rather concentrated gold solution. Then dry upon a sand-bath in a Rose porcelain crucible and by means of a porcelain tube pass pure hydrogen into the crucible while heating strongly by degrees over a free flame. Complete reduction of gold chloride requires about 15 minutes and the asbestos in part is coated with lustrous, finely divided metallic gold. Wash first with dilute hydrochloric acid, then with water and dry. Prepare the filter-amalgamation tube in the following manner. First put a tight plug of ordinary asbestos into the narrow part

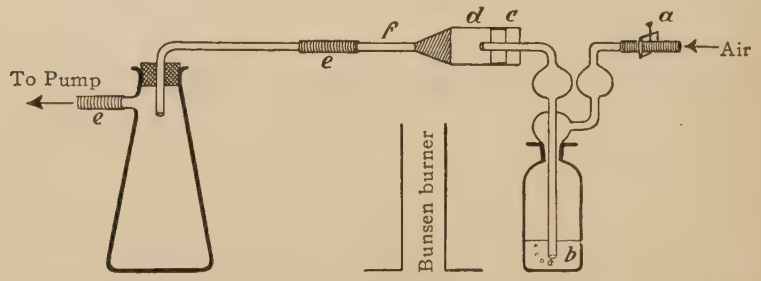


FIG. 51.

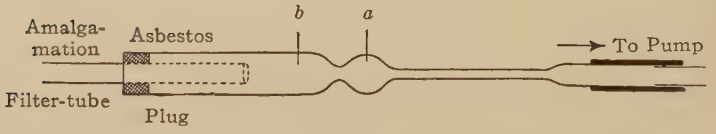


FIG. 52.

FIGS. 50, 51 and 52.—Schuhmacher-Jung apparatus for estimating mercury.

of the tube and over that a layer of gold-asbestos. Then add a layer of finely granulated gold and finally a layer of gold-asbestos. A tube thus prepared will take up very small as well as rather large quantities of mercury. Before it is used for the first time, a tube should be attached to the suction-flask (Fig. 50), washed with hydrochloric acid, water, alcohol and ether, and ignited in a current of air.

Gold-asbestos is a very poor conductor of heat. For this reason it should be very strongly ignited in a current of air to decompose the gold amalgam completely and expel all mercury.

Notes.—By exact observance of the details of procedure and careful manipulation, Schuhmacher and Jung obtained accurate and reliable results in cases where a liter of urine contained as low as 1 mg. of mercury. In their more recent reports these authors state that addition of finely granulated gold is not absolutely necessary.

This method obviously is suitable for the quantitative estimation of mercury not alone in urine but in other physiological fluids and materials as well, such as blood, sputum, intestinal and stomach-contents, faeces, etc. Also it may be employed to advantage in forensic-chemical examinations, especially in those instances where presence of organic matter influences or prevents a direct determination of mercury. Werder[1] quite justly has pointed out that the method described does not furnish positive proof that loss upon ignition is due to mercury and not possibly to other substances accidentally present and volatile at the temperature of ignition. A qualitative test for mercury should always accompany the quantitative determination of loss by ignition. The best procedure for this purpose, according to Werder, consists in attaching to the filter-amalgamation tube an additional tube of the form shown in Fig. 52. Connection may be best made by means of asbestos wicking tamped in tightly. Drive the mercury mirror with heat into the bulb (a) of the second tube. Then cut off the latter at (b) and introduce a small particle of iodine into the bulb. With gentle heat the mercury sublimate may be transformed into a more or less distinct coating of red mercuric iodide. This arrangement also admits of complete expulsion of the mercury deposit from the filter-amalgamation tube which in the earlier form of apparatus was not possible until the rubber connection with the pump had been broken.

Beckers Modification of Farup's Method[2]

Contrary to Schuhmacher and Jung, Beckers found that Farup's method gave results for mercury nearly the same as those given by the method of Schuhmacher and Jung.

Procedure.—Add 3–4 cc. of concentrated hydrochloric acid to a liter of urine and warm upon the water-bath to 70–80° in a strong-wall, roomy flask under a short reflux. Then add about 6 grams of zinc dust, shake well for 2 minutes, and allow the mixture to stand for several hours. After the slightly cloudy liquid has cooled and settled, filter by suction through a filter-disc not too thin and prepared from silky asbestos. At the end of filtration the under surface of the asbestos mat should not have a gray color due to some of the metallic powder sucked through but be perfectly white. Return asbestos and zinc dust without loss to the flask containing most of the zinc dust. Wash off any metallic particles adhering to the sides of the funnel with 80 cc. of dilute hydrochloric acid (1:1). Add 3 grams of potassium chlorate, again connect the flask with the reflux, and

[1] J. Werder: Quantitative Estimation of Mercury in Urine. Zeitschr. f. analyt. Chem. 39 (1900), 358.

[2] W. Beckers: Comparative Determinations of Mercury in Urine by the Methods of Farup and Schuhmacher-Jung. Arch. d. Pharm. 251 (1913), 4.

heat upon the water-bath until everything is in solution. Filter the cold solution through hard paper into a 200 cc. flask. Warm the filtrate, which is green from presence of chlorine, to about 60° and add 15–20 cc. of freshly prepared stannous chloride solution, a quantity in excess of that required to discharge the green color. Mercury is thus precipitated as fine droplets that impart a grayish color to the liquid. Cool to 40° and filter through the filter-amalgamation tube which is an ordinary Soxhlet reduction-tube, containing silky asbestos at the bottom and over that a layer of gold-asbestos 10 mm. thick. After complete filtration, wash in succession with dilute hydrochloric acid, alcohol and ether, using each liquid three times. Continue suction for 5 minutes and finally draw air through the tube for 25–30 minutes. This takes place toward the reduced part of the tube. In a single analysis a layer of gold-asbestos only 1–2 mm. in depth is colored gray. Mercury possibly vaporized by the air-current must therefore pass through a still thicker layer of unamalgamated gold-asbestos. Unquestionably mercury is so firmly held that there is no reason to fear loss during drying.

Notes.—Beckers determined mercury both by the Farup and the Schuhmacher-Jung method in a large number of urines from patients who had been subjected to treatment with blue ointment. The results obtained showed good agreement. So both methods are equally serviceable. He used a chemically pure zinc dust supplied by E. Merck for analytical purposes but not until he had found it absolutely free from mercury. In preparing his filters he used pure white, long-fibre, silk asbestos also supplied by E. Merck. This was not used without first being purified by treatment with hydrochloric acid. After passing a current of air through to expel ether present, Beckers departed from the procedure of Farup by first drying to constant weight and then strongly igniting to volatilize mercury. The difference between the two weighings represents the quantity of mercury in 1000 cc. of the given urine.

Colorimetric Estimation of Mercury

(Autenrieth and Montigny[1])

Inasmuch as only very minute quantities of mercury, following treatment with ointment or poisonings, are eliminated in urine (frequently only fractions of a mg. in the total urine for 24 hours), colorimetric methods are especially suitable for such cases. Autenrieth and Montigny employ the well-known property possessed by mercuric sulphide of forming clear, yellowish brown to pure brown colloidal solutions, when formed by hydrogen sulphide water in very dilute solutions containing mercury. To prevent mercuric sulphide as much as possible from flocking out, they add a clear 1 per cent. gelatine solution as a protective colloid. Colloidal solutions of mercuric sulphide prepared in this manner often remain perfectly clear for many hours and are very well suited for colorimetric examination. This method of estimation has been worked out for the Autenrieth-Koenigsberger colorimeter. This apparatus calls for a comparison-wedge, filled with an artificial color solution that is permanent and calibrated once for all by means of a solution of mercuric chloride of known strength. A calibration-curve may then be constructed from the results of the several readings. The determination of

[1] W. Autenrieth and W. Montigny: Colorimetric Determination of Mercury in Urine. Münch. med. Wochenschr. Nr. 32 (1920), 928.

mercury by this method also requires preliminary destruction of organic matter in the urine by means of hydrochloric acid and potassium chlorate. To precipitate the last traces of mercury from the solution, which is always very dilute, the latter following destruction of organic matter is treated first with zinc chloride and then with excess of sodium acetate. Treatment with hydrogen sulphide then causes precipitation of mercuric sulphide together with considerable zinc sulphide, the latter carrying down traces of the former that may be present. This operation is known as concentration by precipitation.

Procedure.—According to the quantity of mercury suspected present, take 800, 1000 or 1200 cc. of urine for a single determination. Pour the measured volume of urine into a roomy flask, adding first 10–20 cc. of concentrated hydrochloric acid and then 6, 12 or 15 grams of potassium chlorate. Next heat under a reflux for 4–6 hours upon a water-bath, or for 1–1.5 hours over a free flame, with frequent shaking. As a result of this treatment, the color of the urine is nearly discharged. Filter and add to the filtrate somewhat more than the quantity of crystallized sodium acetate calculated for the hydrochloric acid used. If, for example, 20 cc. of 38 per cent. hydrochloric acid are used, take 35 grams of $Na.C_2H_3O_2.3\ H_2O$. Add also 0.02–0.03 gram of zinc chloride, heat nearly to boiling, and pass hydrogen sulphide into the hot solution for about half an hour. Since free chlorine usually remains in this urine mixture, following destruction of organic matter, sulphur is precipitated in abundance. The latter like zinc sulphide can carry down mercuric sulphide colloidally dissolved. Two hours later, or better on the following day, collect this precipitate by suction upon a filter-plate with asbestos mat and wash several times with hydrogen sulphide water. This filtration and washing of the precipitate take only a few minutes. Usually the filtrate is at once obtained perfectly clear. Should it be turbid, however, it must again be passed through the asbestos filter. Then transfer precipitate and asbestos without loss to a rather small porcelain dish and stir with about 5 cc. of hot dilute hydrochloric acid in which a few crystals of potassium chlorate are dissolved. Allow free chlorine to evaporate as completely as possible, then remove it entirely with a little alcohol which should discharge the color of the liquid. Filter the solution into a rather small graduate, allow it to drain well, and repeatedly wash the asbestos, pressing it with a glass rod, with small quantities of hot water, not allowing the total filtrate to amount at most to more than 20 cc. Shake this filtrate well. It should be clear and colorless. Measure 8 cc. into a 10 cc. graduate, add 1 cc. of clear 1 per cent. gelatine solution and 1 cc. of strong hydrogen sulphide water, and shake. After 3–5 minutes determine the color strength of the brownish solution. For this purpose pour a test-portion into the small trough of the colorimeter. Adjust the calibrated comparison-wedge and establish the point at which color-strength is the same as that of the solution in the trough. Note the reading on the scale of the colorimeter and ascertain from the calibration-curve the quantity of mercury corresponding to the reading.

Example.—Urine taken = 500 cc. Volume of solution of mercuric sulphide precipitate = 20 cc. Solution for colorimetric determination = 8 cc. Reading on scale for equal color strength 82 = 0.4 mg. of mercury. So 20 cc. of solution, and therefore 500 cc. of urine taken, must have contained: $2.5 \times 0.4 = 1$ mg. of mercury.

Notes.—In addition to mercury, obviously the solution should not contain such metals as lead, copper, silver and bismuth, for they too are precipitated from acid solution by hydrogen sulphide. Moreover, the possibility of error creeping in should not be overlooked, especially when experiments upon mercury are carried on in a laboratory. Impurities may come from bottles in which the urine was collected, from glass apparatus (reflux, corks, etc.) but especially from reagents (hydrochloric acid, sodium acetate, potassium chlorate, zinc chloride). Without exception every new reagent in acetic acid solution should be tested for purity with hydrogen sulphide. A blank test with about 800 cc. of mercury-free urine should not be omitted! The use of mercury and salvarsan together has raised the question as to whether presence of arsenic has any influence upon the colorimetric method. The answer is negative, for after thorough treatment with hydrochloric acid and potassium chlorate arsenic is always in solution as arsenic acid, H_3AsO_4, and in this condition it is not precipitated in the cold by hydrogen

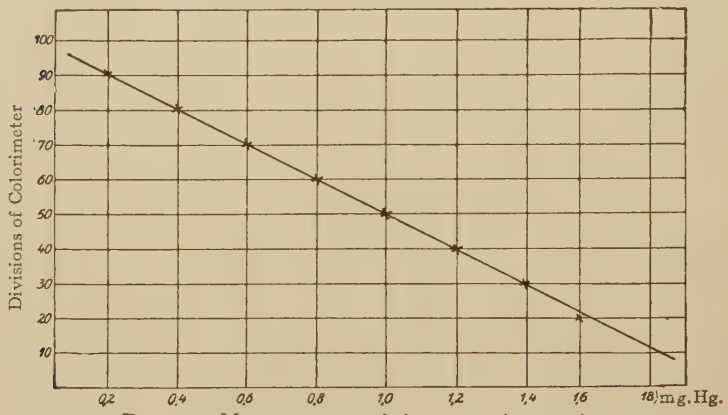


FIG. 53.—Mercury curve of the comparison-wedge.

sulphide, at least not if the hydrochloric acid solution to be examined colorimetrically is treated with hydrogen sulphide water and not allowed to stand longer than 5 minutes. There is also a possibility of a yellowish brown color in the solution of the sulphides of mercury and zinc in hydrochloric acid containing potassium chlorate. In this case color is due to incomplete destruction of organic matter in the urine. The solution in that event should be heated in a small Erlenmeyer flask with a little potassium chlorate and hydrochloric acid until the brown pigment is destroyed. To prevent vaporization of mercuric chloride, connect the flask with an air-cooler, or set in the mouth a glass funnel sealed at the end and filled with water. Asbestos required for the filter-mats should be warmed with hydrochloric acid and a little potassium chlorate and finally washed with hot water. An extraction of asbestos treated in this manner should give no coloration with hydrogen sulphide water. The 1 per cent. gelatine solution made with hot water should be clear and develop no color with hydrogen sulphide water.

Calibration of Comparison-wedge.—To calibrate the comparison-wedge used in the colorimetric determination of mercury, dilute in a graduate varying quanti-

ties, i.e., 1, 2, 3, 4, etc., cc. of a mercuric chloride solution, containing 0.2 mg. of mercury per cc. In each case bring the volume to 9 cc. with water and then always add 1 cc. of strong and absolutely clear hydrogen sulphide water. After 5 minutes establish for each the point of equal color-strength (see above) and take the reading on the scale. Then record on coordinate paper the quantities of mercury in mg. upon the abscissae and upon the ordinates the colorimeter readings for equal color strength with the comparison-wedge. The calibration-curve of the comparison-wedge is obtained by connecting the points of intersection. As is evident from the curve above, this particular comparison-wedge permits the colorimetric determination of quantities of mercury ranging from 0.02–1.6 mg. after dilution in each instance to 10 cc. in the manner described.

III. Estimation of Mercury in Corrosive Sublimate Tablets and Officinal Mercurial Ointments

1. Corrosive Sublimate Tablets

Officinal corrosive sublimate tablets contain approximately 50 per cent. of mercuric chloride.

Following addition of an excess of potassium iodide and sodium hydroxide solution to a solution of mercuric chloride, formaldehyde will reduce potassium-mercuric iodide formed (a) to metallic mercury (b) which, after acidification with acetic acid, is dissolved by excess of 0.1 n-iodine solution (c). Excess of the latter is determined in the usual way with 0.1 n-thiosulphate solution (d).

(a) $HgCl_2 + 4KI = 2KCl + K_2(HgI_4) = K_2(I_2HgI_2)$

(b) $K_2(I_2HgI_2) + H_2O + CH_2O = 2KI + Hg + 2HI + CH_2O_2$

(c) $Hg + I_2 + 2KI = K_2(HgI_4)$

(d) $2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$

Formic and hydriodic acids of the reaction are of course converted to sodium salts by sodium hydroxide solution.

Procedure.—Dissolve in water two 1 gram tablets, or one 2 gram tablet, and bring the volume of this solution to 100 cc. Dissolve 1 gram of potassium iodide in 20 cc. of this solution = 0.4 gram of corrosive sublimate tablet. Add 10 cc. of sodium hydroxide solution, 3 cc. of formaldehyde solution, 10 cc. of water, and after 1 minute acidify with 25 cc. of dilute acetic acid. Dissolve the precipitated mercury in 25 cc. of 0.1 n-iodine solution and titrate excess of iodine with 0.1 n-thiosulphate solution, of which at least 10.4 cc. are used, using starch solution as indicator. Since commercial corrosive sublimate tablets often differ in weight, they should be ground in a mortar and exactly 2 grams taken for this examination.

Calculation.—The mercury reduced from 0.4 gram of corrosive sublimate tablets = 20 cc. of solution has combined with 25–10.4 = 14.6 cc. of 0.1 n-iodine solution. According to the above equations, 1 atomic weight of iodine corresponds to 0.5 atomic weight of mercury and so to 0.5 molecular weight of mercuric

chloride = 0.5 × 271 = 135.5 grams of $HgCl_2$. Thus 1000 cc. of 0.1 n-iodine solution = 13.55 grams and 1 cc. of 0.1 n-iodine = 0.01355 gram of $HgCl_2$.14.6 cc. of 0.1 n-iodine solution = 14.6 × 0.01355 = 0.19783 gram of $HgCl_2$. This quantity of mercuric chloride is contained in 0.4 gram of tablet, corresponding to at least 250 × 0.19783 = 49.45 per cent. of mercuric chloride.

2. Unguentum Hydrargyri Album = Ammoniated Mercury Ointment

The white precipitate in the ointment is brought into solution by means of dilute hydrochloric acid:

$$NH_2HgCl + 2HCl = HgCl_2 + (H_4N)Cl$$

and mercuric chloride in solution is determined quantitatively in the manner described for corrosive sublimate tablets.

Procedure.—Warm 5 grams of ammoniated mercury ointment upon the water-bath for 10 minutes in a flask with 25 grams of dilute hydrochloric acid (sp. gr. 1.061–1.063 = about 12.5 per cent. of HCl) and shake frequently. Then add about 30 cc. of water and allow to cool. Pour the cold solution into a 100 cc. graduated flask, rinsing the vaseline layer repeatedly with water and bringing the volume of the liquid to 100 cc. Add 1 gram of potassium iodide and 10 cc. of sodium hydroxide solution to 25 cc. of this solution in a glass-stoppered flask. Then add 3 cc. of formaldehyde solution, 10 cc. of water, and after 1 minute acidify with 25 cc. of dilute acetic acid. Dissolve the precipitated mercury in 20 cc. of 0.1 n-iodine solution and titrate excess of iodine with 0.1 n-sodium thiosulphate solution, using starch solution as indicator. This should require 10.1 cc. of 0.1 n-thiosulphate solution.

Calculation.—According to the equations above, the equivalent weight of mercury precipitated is equal to half its molecular weight = 0.5 ($H_2N.HgCl$) = 0.5 × 251.4 = 125.7. Hence 1000 cc. of 0.1 n-iodine solution correspond to 12.57 grams and 1 cc. corresponds to 0.01257 gram of mercury precipitate. According to the requirements of the German Pharmacopoeia, metallic mercury precipitated from 25 cc. of the hydrochloric acid solution = 1.25 gram of ammoniated mercury ointment combined with 20 — 10.1 = 9.9 cc. of 0.1 n-iodine solution. This represents 9.9 × 0.01257 = 0.1245 gram of white precipitate, corresponding to a content in the ammoniated mercury ointment of 80 × 0.1245 = approximately 10 per cent. of white precipitate.

3. Unguentum Hydrargyri Cinereum = Mercury Ointment and Unguentum Hydrargyri Rubrum = Mercury Oxide Ointment

The mercury content of these two preparations is determined by means of the reaction taking place between mercuric nitrate and ammonium sulphocyanate in presence of ammonium ferric sulphate as indicator. The red color first appearing is due to ferric sulphocyanate. It continues to disappear and does not become permanent

until all mercury is converted into mercuric sulphocyanate, $Hg(SCN)_2$:

(a) $(H_4N)Fe(SO_4)_2 + 3(H_4N)SCN = Fe(SCN)_3 + (H_4N)_2SO_4$
(b) $2Fe(SCN)_3 + 3Hg(NO_3)_2 = 3Hg(SCN)_2 + 2Fe(NO_3)_3$

In this determination of mercury, hydrochloric acid and chlorides should not be present!

(a) **Mercury Ointment.**—Heat 2 grams of mercury ointment upon the water-bath for about 10 minutes in a wide-neck flask with reflux. As soon as no more mercury particles can be seen in the mixture, add 25 cc. of water by rinsing the reflux and heat again until the fat layer has separated clear. Pour this solution when cold through a cotton plug into a 100 cc. graduated flask, break up the fat layer, and wash it and the flask 4–5 times with about 5 cc. of water. Add sufficient potassium permanganate solution to the total aqueous solution to produce a permanent red color, or separation of brown flocks, and decolorize or clear the mixture with ferrous sulphate solution. Then bring the solution to the mark. Treat 25 cc. of this solution = 0.5 gram of mercury ointment with 2 cc. of ammonium ferric sulphate solution and sufficient 0.1 n-ammonium sulphocyanate solution to produce a permanent red-brown color. This should require 15 cc. of 0.1 n-ammonium sulphocyanate solution.

Calculation.—According to the above equation, the equivalent weight of mercury corresponds to half its atomic weight = 100. So 1000 cc. of 0.1 n-ammonium sulphocyanate correspond to 10 grams and 1 cc. to 0.01 gram of mercury. The appearance of the end-reaction should require 15 cc. of 0.1 n-ammonium sulphocyanate = $15 \times 0.01 = 0.15$ gram of mercury in 0.5 gram of gray mercury ointment, corresponding to a content of $200 \times 0.15 = 30$ per cent. of mercury.

(b) **Oxide of Mercury Ointment.**—Heat 5 grams of this ointment upon the water-bath with 20 cc. of nitric acid in a wide-neck flask upon which a funnel is set and shake frequently. As soon as the red color of the ointment has completely disappeared, add 25 cc. of water by rinsing off the funnel and heat again until the fat layer has settled clear. From the point "Pour this solution when cold" the procedure is the same as for gray mercury ointment, except that 50 cc. instead of 25 cc. of the filtered solution = 2.5 grams of oxide of mercury ointment are used in the titration. For the end-reaction 23.1 cc. of 0.1 n-ammonium sulphocyanate solution should be required.

Calculation.—The equivalent weight of mercuric oxide is equal to half its formula weight = 0.5 HgO = 108. So 1000 cc. of 0.1 n-ammonium sulphocyanate = 10.8 grams and 1 cc. = 0.0108 gram of mercuric oxide. And 23.1 cc. of 0.1 n-sulphocyanate solution correspond to $23.1 \times 0.0108 = 0.2495$ gram of mercuric oxide, corresponding to a content of $0.2495 \times 40 = 10$ per cent. of mercuric oxide.

Notes.—Addition of potassium permanganate to the solution of mercuric nitrate is for the purpose of oxidizing mercurous salt that may be present to mercuric salt and dissolved nitrous to nitric acid. The color due to excess of potassium permanganate is then discharged by ferrous sulphate.

Quantitative Estimation of Silver in Organs and Urine

The best and only practical method of determining silver in organs, such as liver, spleen, kidneys and brain, as well as in urine is that described by Lehmann.[1] By this method the organ is first well dried and then finely triturated. After fusion with sodium carbonate and potassium nitrate, the cold melt is extracted with water and any undissolved residue, that is, metallic silver, is well washed and finally dissolved in hot, moderately dilute nitric acid. This solution is evaporated upon the water-bath to dryness. The residue is dissolved in hot water with addition of 2 drops of nitric acid, silver is precipitated from this solution as chloride and finally weighed. In the precipitation of silver a large excess of hydrochloric acid should be avoided, because silver chloride is appreciably soluble in this acid. In the case of urine, it should be mixed with sodium carbonate and potassium nitrate, evaporated to dryness, the residue fused, and the melt further treated in the manner described.

B. ORGANIC CHEMICAL EXAMINATIONS

Hydrocyanic Acid

1. Colorimetric Estimation of Minute Quantities of Hydrocyanic Acid

(Autenrieth and Quantmeyer[2])

The guaiac tincture-copper sulphate test of Schönbein may be used to excellent advantage in the colorimetric estimation of very small quantities of hydrocyanic acid, since a blue color is distinctly visible even in concentrations of $1:10^7$ and less of this acid. This color is due to oxidation of guaiaconic acid in guaiac resin to guaiac-blue through the influence of cupric cyanide, $Cu(CN)_2$, which acts as a carrier of oxygen as shown in the following equations:

(a) $CuSO_4 + 2HCN = Cu(CN)_2 + H_2SO_4$
(b) $4Cu(CN)_2 + 2H_2O = 4CuCN + 4HCN + O_2$ and
(c) $6Cu(CN)_2 + 3H_2O = 6CuCN + 6HCN + O_3$

Use of 50 per cent. alcohol makes it possible to hold in solution the small quantity of guaiac resin as well as the trace of copper sulphate, so that the color solutions obtained are pure blue and absolutely clear. The reaction is carried out in neutral, or faintly acetic acid solution, but never in alkaline solution. The blue color is immediately developed to a maximum and should be determined colorimetri-

[1] V. Lehmann: Experimental Investigation as to the Best Method of Detecting Lead, Silver and Mercury in the Animal Organism after Poisoning. Zeitschr. f. physiolog. Chem. 6 (1882), 19.

[2] As yet unpublished by the authors.

cally within 3 minutes, otherwise on longer standing the intensity of color diminishes.

Requirements.—In carrying out this colorimetric estimation of hydrocyanic acid, the following solutions are needed: 1. Approximately 5 per cent. alcoholic tincture of guaiac, freshly prepared; 2. Aqueous 1 per cent. copper sulphate solution; 3. Pure 50 per cent. alcohol; 4. A potassium cyanide solution of known strength.

Calibration of Comparison-wedge

The glass-stoppered wedge of the Autenrieth-Koenigsberger colorimeter (see page 471) is first filled with a very dilute copper tetramine sulphate solution (copper sulphate + excess of ammonia) which has the same shade of color as the hydrocyanic acid color solution to be compared. The comparison-wedge is calibrated with an aqueous solution of purest potassium cyanide, the potassium cyanide content of which has previously been accurately determined gravimetrically, or volumetrically by Volhard's method. If after each test the copper tetramine solution is poured from the comparison-wedge into a dark, glass-stoppered bottle, it will retain its strength of color longer, so that one calibration of the comparison-wedge filled with this solution is sufficient. Then weigh a quantity of potassium cyanide that shall represent 2.41 grams of absolute KCN, corresponding to 1 gram of HCN, bring this with water to a volume of 1000 cc., and dilute 10 cc. of this solution again with water to 1000 cc. One cc. of the latter solution corresponds to 0.01 mg. of HCN. It is used in calibrating the comparison-wedge and should be freshly prepared for this purpose. Put 5 cc. of purest alcohol into a 10 cc. graduate with 3 drops each of guaiac tincture and copper sulphate solution. Mix thoroughly and with a capillary pipette run out different measured volumes of potassium cyanide solution, that is, 0.2–1.6 cc. (1 cc. = 0.01 mg. HCN), bring to the 10 cc. mark with water and shake. Pour a test portion of the blue color-solution thus prepared into the glass trough of the colorimeter and at once determine in the usual manner by adjusting the position of the comparison-wedge the point at which the color-strength of the two solutions is equal. Each time take this reading several times and the average of the several readings is taken as the basis of the calibration. In one calibration the following values were obtained for six different concentrations of hydrocyanic acid:

Potassium cyanide solution in cc.....	0.2	0.4	0.6	0.8	1.0	1.2
HCN in thousandths of a mg. in 10 cc. of color-solution.....	2	4	6	8	10	12
HCN in thousandths of a mg. in 1 cc. of color-solution.....	0.2	0.4	0.6	0.8	1.0	1.2
Scale reading.....	89	79	68	56	46	34

From these figures the calibration-curve of the comparison-wedge may be constructed as shown in Fig. 54.

Procedure.—As occasion may require, distillates, officinal hydrocyanic acid, or other solutions of this acid, should be so diluted to be within the reading limits of the comparison-wedge. In other respects the method is the same as that described for the calibration. Oil of bitter almond water, or cherry laurel water, should first be diluted one hundred times. Then in order to bring about hydroly-

sis of benzaldehyde cyanhydrine, add 1 drop of sodium hydroxide solution to 1 cc. of this diluted solution, and render faintly acid with acetic acid.

In an examination of 100 cc. of freshly distilled Black Forest cherry brandy 34 mg. of total hydrocyanic acid were found. But in a colorimetric determination without first hydrolyzing with sodium hydroxide only 20 mg. of free hydrocyanic acid were found in the same quantity of cherry brandy.

A sample of plum brandy gave considerably lower results, that is, 10 cc. were found to contain 1.0 mg. of free and 1.2 mg. of total hydrocyanic acid.

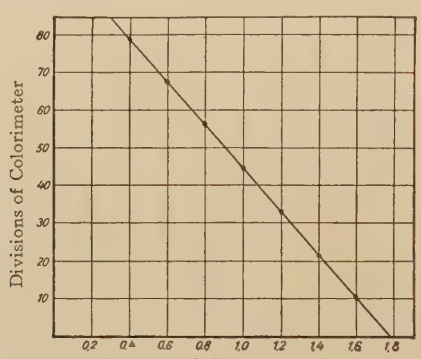


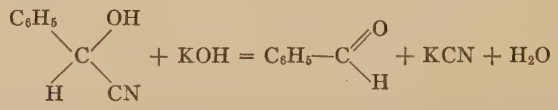
FIG. 54.—Hydrocyanic acid curve for comparison-wedge.

Notes.—It should be borne in mind that the guaiac-copper sulphate test is not characteristic of hydrocyanic acid. More detailed statements with regard to this matter are given on page 28.

2. Estimation of Total Hydrocyanic Acid in Bitter Almond Water

(German Pharmacopoeia)

About 20 per cent. of the hydrocyanic acid in Bitter Almond Water is in the form of free acid and about 80 per cent. as benzaldehyde cyanhydrine, or the nitrile of mandelic acid. To determine total hydrocyanic acid in this preparation, benzaldehyde cyanhydrine should first be hydrolyzed by sodium hydroxide solution, or according to directions given in the German Pharmacopoeia by aqueous ammonia:



Excess of ammonia condenses with benzaldehyde after hydrolysis and forms hydrobenzamide:

$$3C_6H_5.CHO + 2H_3N = (C_6H_5.CH)_3N_2 + 3H_2O.$$

Hydrocyanic acid is then titrated in this ammoniacal solution with 0.1 n-silver nitrate solution with potassium iodide as indicator. As the silver nitrate solution is added drop by drop, a yellowish white milkiness due to silver iodide appears

temporarily. This disappears with shaking, or stirring, as long as the solution contains ammonium cyanide. But as soon as all hydrocyanic acid has been converted into complex ammonium silver cyanide, which is easily soluble, this milkiness becomes permanent:

$$\text{(a)} \qquad AgNO_3 + KI = AgI + KNO_3$$
$$\text{(b)} \quad AgI + 2(H_4N)CN = (H_4N)(Ag(CN)_2) + (H_4N)I$$

Procedure.—Dilute 25 cc. of Bitter Almond Water with 100 cc. of water. Add 2 cc. of potassium iodide solution (10 per cent.) and 1 cc. of ammonia. Then 4.5–4.8 cc. of 0.1 n-silver nitrate solution should be required to produce a permanent milkiness, corresponding to a content of 0.099–0.107 per cent. of hydrocyanic acid.

Calculation.—On the basis of equation (b) above, 1 molecule of silver nitrate corresponds to 2 molecules of hydrocyanic acid = 54.04. So 1000 cc. of 0.1 n-silver nitrate solution correspond to 5.404 grams and 1 cc. = 0.005404 gram of hydrocyanic acid. Estimation of total hydrocyanic acid in cherry-laurel water = Aqua Laurocerasi is conducted in precisely the same manner as described for bitter almond water.

Detection of Methyl Alcohol in Brandies and Other Liquids Containing Alcohol

1. By Detection of Formaldehyde from Methyl Alcohol

(a) **Method of Fendler and Mannich.**[1]—By means of a small flame heat 10 cc. of the given liquid to boiling very slowly and carefully in a 50 cc. flask. Attached to the latter, is a perforated rubber stopper through which passes a glass tube doubly bent at right angles and serving as a dephlegmator and cooler. The flask stands upon a double wire gauze. A small graduate divided into 0.1 cc. acts as receiver of the distillate. Distil off exactly 1 cc. and conduct distillation so that 4–5 minutes are required for the distillate to pass over. If distillation is too rapid, the distillate gets hot and then loss of methyl alcohol may occur.

Mix this distillate with 4 cc. of dilute sulphuric acid (2 per cent.) and transfer to a test-tube. Immerse the latter in cold water, shake constantly, and gradually introduce 1 gram of finely powdered potassium permanganate. As soon as the violet color from the last portion has disappeared, filter into a test-tube through a small, dry paper. Gently boil for 20–30 seconds the filtrate which is usually reddish. Keep well cooled and mix 1 cc. of the colorless liquid in a test-tube with 5 cc. of pure concentrated sulphuric acid. Then add to the cooled mixture 2 5 cc. of a freshly prepared solution of 0.2 gram of morphine hydrochloride in 10 cc. of concentrated sulphuric acid. Since these liquids are mixed with difficulty by shaking, stir carefully with a glass rod. After 20 minutes standing at room temperature, observe the color of the mixture. If the original liquid contained at least 0.5 per cent. of methyl alcohol, the liquid will acquire an intense violet to reddish violet color. Yellowish and brownish colorations are to be disregarded.

Notes.—In order to become familiar with this reaction, it is advisable to prepare a mixture of 99 volumes of alcohol and 1 volume of methyl alcohol. Treat 1

[1] F. Fendler and C. Mannich: Detection of Methyl Alcohol in Spirituous Preparations. Arb. a.d. Pharm. Institut d. Universität Berlin 3 (1906), 243.

cc. of this mixture precisely in the manner described for the distillate. If in the examination of an alcoholic preparation a color is obtained, that is as strong or stronger than that given by the 1 per cent. methyl alcohol, the presence of methyl alcohol is definitely proved.

Juckenack states with regard to the delicacy of this test that by careful observance of directions a brandy containing 1 per cent. of methyl alcohol will give a distinct violet color in 2–3 minutes. Therefore it does not seem advisable, when it is a case of determining whether methyl alcohol has been used as a substitute for alcohol, to take into consideration colors that may appear after a longer time. Especial care is required in judging rum and some brandies prepared from fruits, since these spirituous liquors apparently may normally contain traces of methyl alcohol, or of a methyl alcohol compound. A rum essence, for example, gave Juckenack a distinct test for methyl alcohol.

Wirthle[1] substitutes the morphine salt itself for the solution of 0.05 gram of morphine hydrochloride in concentrated sulphuric acid. Out of several dozen samples of spirituous liquors tested in this manner, among the number being ordinary potato whiskey, crême de menthe and other aromatic liqueurs, arrac, rum, imitation rum and arrac, and finally cognac, only a single test gave a very faint reaction, and the rest only a red coloration.

(b) **Wirthle's Method.**—To determine the alcoholic content of spirituous liquor, distil 100 cc. The distillate, amounting to 45, or at most 50 cc., is then fractioned. When a considerable quantity of methyl alcohol is present, a lower boiling-point will be noticed during fractionation. Collect 15 cc. as the first fraction and re-fraction that, collecting only 3 cc. Add 4 cc. of dilute sulphuric acid to 1 cc. of this distillate, cool well, shake vigorously, and gradually add in small portions 1 gram of finely powdered potassium permanganate. In other respects the method is identical with that of Fendler and Mannich, with this exception that Wirthle sprinkles 0.05 gram of morphine hydrochloride upon the well cooled mixture of 1 cc. of the oxidized liquid and 5 cc. of concentrated sulphuric acid. As far as delicacy of this test is concerned, if directions are followed exactly, a brandy containing 1 per cent. of methyl alcohol will as a rule give a violet color in 2–3 minutes. Therefore it is well to disregard a test appearing after a longer time, at least when it is a question of deciding whether methyl alcohol has been substituted for alcohol.

Notes.—Wirthle in his experiments has also made a comparison of this test with the Rimini test, recommended by Arnold and Mentzel for detection of formaldehyde in milk, and with the hexamethylene-tetramine test adopted by the meat inspection act for detection of formaldehyde in brandies. Although the former test is well suited for detection of formaldehyde in milk, it is too sensitive to use in testing spirituous liquors for methyl alcohol, for Wirthle found that a large number of the samples of cognacs and liqueurs examined gave a positive result when certainly neither methyl alcohol nor denatured alcohol had been added. Just the reverse is true of the hexamethylene-tetramine test. The original liquid must contain several decigrams of methyl alcohol before it will give the micro-crystalline reaction.

[1] F. Wirthle: Detection and Estimation of Methyl Alcohol. Zeitschr. f. Unters, d. Nahrgs.-u. Genussm. 23 (1912), 16; 24 (1912), 14.

2. By Oxidation of Methyl Alcohol to Formaldehyde by Potassium Dichromate and Sulphuric Acid and Condensation of Aldehyde with Dimethyl-Aniline

(Trillat[1] and Wolff[2])

Upon oxidation with potassium dichromate and sulphuric acid, pure alcohol yields acetic aldehyde, ethylal, $CH_3.CH(OC_2H_5)_2$, and acetic acid as the principal products. Under the same conditions methyl alcohol yields methylal, $CH_2(OCH_3)_2$, but chiefly formaldehyde. The two distillates may be differentiated by their content of methylal or ethylal. Both form condensation-products with dimethyl-aniline. Methylal, derived from methyl alcohol, forms tetramethyl-diamino-diphenyl methane:

$$CH_2(OCH_3)_2 + 2HC_6H_4.N(CH_3)_2 = 2CH_3.OH + CH_2(C_6H_4.N(CH_3)_2)_2$$

which may be converted into a blue dyestuff, giving a blue color that can be detected in a dilution of 1:200,000. On the other hand, the condensation of the oxidation-product of alcohol with dimethyl-aniline gives a blue color that quickly disappears upon warming. Heat renders the blue color produced by the derivative of methyl alcohol more intense. By this method 1 per cent. of methyl alcohol may be detected in alcohol. The dimethyl-aniline used should be very carefully purified and after condensation any excess should be completely removed by steam distillation.

Procedure of Wolff.—Dissolve 15 grams of potassium dichromate in 130 cc. of water, adding first 70 cc. of pure dilute sulphuric acid (1:5) and then 10 cc. of the alcohol. Distil after 20 minutes, rejecting the first 25 cc. of distillate which consists chiefly of acetic aldehyde. Add 1 cc. of pure dimethyl-aniline to 50 cc. of the next 100 cc. of distillate and allow the mixture to stand for 24 hours at room temperature in a well stoppered flask. Then add 4–5 drops of dilute phenolphthalein solution and exactly neutralize with sodium hydroxide solution. Add a few pieces of pumice-stone and distil off 30 cc. Add to the distillation-residue 25 cc. of water, 1 cc. of acetic acid, and observe whether a blue color appears upon addition of lead dioxide. Presence of methyl alcohol may be safely decided upon only when a blue color, somewhat like the blue of Fehling's solution, appears. Green, yellow-green, or even a blue-green color, is without significance, for such colors are produced by acetic aldehyde.

Notes.—Dimethyl-aniline, according to Robine, should be carefully purified. Allow 500 grams of the commercial product to stand for 1–2 hours in contact with calcium chloride and shake frequently. Then filter and distil, using a still-head and collecting only the portion that goes over at 192°. As proof of purity, warm 1 cc. with 50 cc. of water and 2–3 drops of acetic acid for 2.5 hours at 70–80°. Distil off excess of dimethyl-aniline with steam and treat the residue with lead dioxide as in the test for methyl alcohol. If dimethyl-aniline is found pure, preserve in the dark in amber, glass-stoppered bottles. To assure a characteristic test for methyl alcohol with dimethyl-aniline, any acetic anhydride

[1] A. Trillat: Detection and Estimation of Methyl Alcohol in Alcohol. Compt. rend. 127 (1898), 232.

[2] J. Wolff: Improvement in theMethod of Trillat for Detecting Methyl Alcohol in Alcohols. Ann. Chim. anal. appl. 4 (1899), 183.

present should be previously removed. Finally, failure to remove completely excess of dimethyl-aniline may easily be the means of causing doubtful color reactions.

Purity of lead dioxide should also be tested. The quantity of this oxidizing agent used materially influences the distinctness of the end-reaction. In certain instances methyl alcohol has been detected in the residue left after distilling off 50 cc., so that it is not advisable to limit the test only to the 50 cc. distilled off. With proper observance of all these sources of error, Robine was able to detect as low as 0.1 per cent. of methyl alcohol in vinegar.

Detection of Methyl Alcohol in Presence of Caramel or Sugar

Wolff has found that alcohol, free from methyl alcohol and containing caramel or sugar, on treatment with chromic acid yields on subsequent distillation polymerization products of formaldehyde. These may be recognized by the blue color with dimethyl-aniline and lead dioxide. Further investigation showed that levulose is the only sugar that gives this reaction. Since this test may also be used in examining alcohol for presence of methyl alcohol, brandies and liqueurs, that contain cane-sugar, invert-sugar or caramel, should first be distilled before being examined. The distillate may then be tested for methyl alcohol.

Detection of Methyl Alcohol in Presence of Alcohol

(Denigès)

Denigès,[1] in testing alcohol for methyl alcohol following preliminary oxidation, makes use of the fact that fuchsine-sulphurous acid in strong sulphuric acid solution shows formaldehyde but not acetic aldehyde. To oxidize the alcohols to aldehydes, mix 1 cc. of the sample with 5 cc. of 1 per cent. potassium permanganate solution and 0.2 cc. of pure concentrated sulphuric acid. Oxidation of alcohols to aldehydes is complete in 2–3 minutes. Now add 1 cc. of cold saturated oxalic acid solution (about 8 per cent.), to discharge color due to excess of potassium permanganate and, if necessary, 1 cc. of concentrated sulphuric acid. Then add 5 cc. of fuchsine-sulphurous acid, to remove color from the solution. After 15 minutes at longest a distinct violet color will appear, if the alcohol contains only a thousandth of its volume of methyl alcohol. The delicacy of this method may be considerably increased, if the alcohol is first subjected to fractional distillation. Alcohol is said not to interfere with this test.

Detection of Formaldehyde in Foodstuffs

1. Detection in Meat of Formaldehyde and Substances Giving Formaldehyde

(Revised Analytical Directions of the German Meat Inspection Act of January 30, 1908)

Thoroughly mix 30 grams of comminuted meat with 200 cc. of water and, after 30 minutes standing in a 500 cc. flask, add 10 cc. of 25 per cent. phosphoric acid. Distil off 50 cc. from the mixture, at the same time passing in steam. Filter this

[1] G. Denigès: General Detection of Methyl Alcohol, Particularly in Alcohol. Compt. rend. de l'Acad. des sciences 150 (1910), 832.

distillate. If the meat is unsmoked, mix 5 cc. of distillate in a large test-tube with 2 cc. of fresh (unboiled) milk and 7 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.), containing 0.2 cc. of 10 per cent. ferric chloride solution in 100 cc., and keep at a gentle boil for about half a minute. Preliminary tests should be made to show that the milk contains no formaldehyde and also that it responds to the test when formaldehyde is added. If the meat is smoked, dilute a portion of the distillate with 4 volumes of water and test 5 cc. of this diluted solution as described. If formaldehyde is present, it produces a violet color. If the latter fails to appear, further testing is unnecessary. On the other hand, add ammonia in excess to the remainder of the distillate and evaporate to dryness, keeping the solution alkaline by occasionally adding ammonia. If the quantity of formaldehyde is not too small, characteristic crystals of hexamethylene-tetramine will remain. Dissolve such a residue in about 4 drops of water, place a drop of this solution on a slide, and test with the following reagents:

1. Add a drop of saturated mercuric chloride solution. A crystalline precipitate will appear at once, or after short standing. Radiating stars are soon visible and later octahedrons.

2. Add a drop of potassium mercuric iodide solution and a very small quantity of dilute hydrochloric acid. Yellow stars of hexagonal shape will appear.

Preparation of Potassium Mercuric Iodide Solution.—Add mercuric iodide to 10 per cent. potassium iodide solution, warming and stirring until it no longer dissolves. Filter this solution when cold.

In smoked meat products, presence of formaldehyde is proved only when both tests are given. On the other hand, the test with mercuric chloride is sufficient to establish its presence in unsmoked meat products.

Notes.—Meat in which formaldehyde has been detected by the above directions is to be regarded as having been treated with formaldehyde, or with some substance that gives formaldehyde.

2. Modification of Hehner's Test for Detection of Formaldehyde in Milk and Other Food-stuffs

Mix 5 cc. of milk with 10 cc. of hydrochloric acid (sp. gr. 1.19 = 38.7 per cent.) containing a trace of nitric acid or potassium nitrate. If the milk contains formaldehyde, a fine violet color will appear. This color appears immediately in a dilution of 1:500,000. Other oxidizing agents are milder in their action (Friese[1]).

Rachel[2] has proposed the following modification of this test for detection of formaldehyde in meat, caviar and fish-products. After addition of phosphoric acid, distil with steam. Thoroughly shake in a separatory funnel 1–2 cc. of distillate with 4 cc. of formaldehyde-free milk and 10 cc. of hydrochloric acid (sp. gr. 1.19 = 38.7 per cent.), containing 1 drop of 25 per cent. nitric acid in 300 cc. The mixture at once turns blue, if formaldehyde is present. Since only traces but not larger quantities of formaldehyde give this reaction, it is advisable in case of a negative result to dilute the distillate with water and repeat the test. In

[1] W. Friese: Determination of Formaldehyde in Milk. Arb. a. d. hyg. Institut Dresden 2 (1908), 109.

[2] F. Rachel: Detection of Formaldehyde in Foods. Pharm. Zentralbl. 54 (1913), 759.

order to determine whether free or combined formaldehyde was used as a preservative, another portion of material should be distilled without addition of phosphoric acid. If the distillate now gives a negative test, then hexamethylenetetramine or a similar compound was used as the preservative.

3. Detection of Formaldehyde in Foodstuffs by Fuchsine-Sulphurous Acid

(Fincke[1])

Detection of formaldehyde in foodstuffs usually requires distillation of the material. In most cases the fuchsine-sulphurous acid method makes this unnecessary. This test may be applied to milk direct. Its delicacy is 1:400,000, when 1 cc. of hydrochloric acid is used for 10 cc. of milk. Color should be removed from highly colored liquids, such as red wines, fruit juices, extracts of chopped meats, by preliminary treatment with animal charcoal. Add first the hydrochloric acid (1–2 cc., sp. gr. 1.124 = 25 per cent. to 10 cc. of liquid) required for the reaction and then animal charcoal, warming, if necessary, shaking and filtering. In place of animal charcoal, concentrated mercuric acetate solution may be used for red wines and fruit juices, or mercuric chloride solution for meat extracts. By decolorization the sensitiveness of the test is usually raised to 1:300,000–1:500,000, thereby permitting detection of quantities that frequently cannot be found after distillation.

Procedure.—Mix 10 cc. of the liquid with 1–2 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.) and then add 1 cc. of the reagent.[2] According to the quantity of formaldehyde present, a persistent blue to red-violet color will appear within a few minutes, or 12 hours at latest. Obviously all substances, which like hexamethylene-tetramine and methylal furnish formaldehyde in acid solution, give this test.

Bremer and Beythien[3] found that the fuchsine-sulphurous acid test is not applicable in testing for formaldehyde in chopped meat, since in many cases pure, formaldehyde-free meat treated in th same manner gave a red color that was faint to be sure but recognizable. Hehner's test gave far better results, since it is very sharp and not subject to the influence of most organic compounds. It may be regarded as a method characteristic of formalin.

COLORIMETRIC ESTIMATION OF FORMALDEHYDE

(Autenrieth[4])

Colorimetric methods are especially useful when it is a matter of determining quantitatively minute amounts of substances.

[1] H. Fincke: Detection of Small Quantities of Formaldehyde and Formaldehyde-compounds by Fuchsine-sulphurous Acid. Zeitschr. f. Nahrgs.-u. Genussm. 27 (1914), 246.

[2] See "Preparation of Reagents" page 642.

[3] W. Bremer and R. Beythien: Comparative Value of the Methods Most Used in Detecting Formaldehyde in Chopped Meat. Zeitschr. f. Unters. d. Nahrgs.-u. Genussm. 18 (1910), 733.

[4] From the author's unpublished manuscript.

Autenrieth has adapted the phloroglucinol test to the colorimetric estimation of formaldehyde in urine following administration of hexamethylene-tetramine or formaldehyde solutions. This method obviously may be used in the examination of food-stuffs and beverages, such as chopped meat, sausage, milk, butter and beer, when only formaldehyde added as a preservative is to be estimated. This method has been worked out for the Autenrieth-Koenigsberger wedge-colorimeter (see page 468). As in all cases where this apparatus finds application, a comparison-wedge is required in the colorimetric estimation of formaldehyde. This is filled with an artificial color-solution that is permanent. This solution has the same optical properties as alkaline formaldehyde-phloroglucinol solution. The comparison-wedge is calibrated by means of an extremely dilute formaldehyde solution of known strength and the calibration figures are checked from time to time.

Calibration of Comparison-Wedge and Construction of Calibration-Curve

Very carefully determine by Romijn's iodimetric method (see page 87) the formaldehyde content of commercial formaldehyde solution. Then by dilution

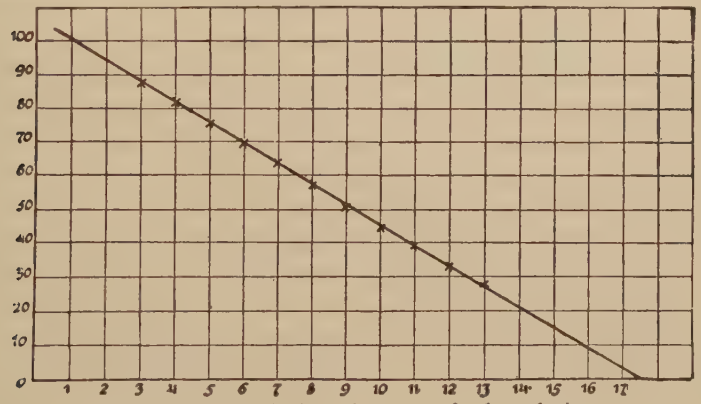


FIG. 55.—Formaldehyde in 10 cc. of color solution.

with water prepare a solution containing 0.05 mg. of formaldehyde in 1 cc. By means of a burette or graduated pipette measure varying quantities of this very dilute formaldehyde solution and dilute in each case with water to 10 cc. Add 1 cc. of alkaline phloroglucinol solution (1 gram of phloroglucinol dissolved in 100 cc. of 10 per cent. sodium hydroxide solution), shake and after 2–2.5 minutes determine in the colorimeter the color-strength of each solution. After lapse of this time the color reaches a maximum. Introduce a portion of the color-solution into the small glass trough of the colorimeter and adjust the comparison-wedge to

the point at which the two solutions have the same color strength. Repeat each reading several times and take the average result. In this manner the following readings were obtained:

Formaldehyde solution....	2	3	4	5	6	7	cc.
Water.....	8	7	6	5	4	3	cc.
Formaldehyde in 10 cc....	0.1	0.15	0.2	0.25	0.3	0.35	mg.
Equal color strength at....	78	69	61	54	47	39	on scale

In each case record upon coördinate paper the quantity of formaldehyde in mg. in 10 cc. of solution upon the abscissae and the colorimeter reading for equal color-strength upon the ordinates. In this way a calibration-curve of the comparison-wedge may be constructed for use in formaldehyde determinations.

This comparison-wedge has a range from 0.05–0.5 mg. of formaldehyde and represents in each case these quantities in 10 cc. of color-solution.

Estimation of Formaldehyde in Urine[1]

Add 30 cc. of 25 per cent. phosphoric acid to 200 cc. of urine and distil in a vigorous current of steam until finally 10 cc. of distillate fails to give a color with 1 cc. of phloroglucinol reagent, at least one that can be determined in the colorimeter. After thorough shaking determine the volume of total distillate. Mix 10 cc. of the latter with 1 cc. of phloroglucinol reagent and after 2 minutes determine color strength in the colorimeter as described above for the calibration of the comparison-wedge. From the calibration-curve the quantity of formaldehyde in 10 cc. of distillate corresponding to the reading may be obtained. The distillate from urine collected after administration of hexamethylene-tetramine usually contains so much formaldehyde that it has to be diluted before readings can be brought within the range of the comparison-wedge. Obviously such dilution should be taken into consideration in the final calculation.

Following internal administration even of considerable quantities of hexamethylene-tetramine by the mouth or by intravenous injection, only a very small part, usually a few mg., is found in urine as free formaldehyde. By means of the phloroglucinol reagent it may be determined direct in the colorimeter without previously distilling the urine. Practically all hexamethylene-tetramine taken into the human body passes unchanged into the urine.

Note.—Formaldehyde distils so slowly with steam that in the procedure described above it is usually necessary to collect 2–2.5 liters of distillate before a test portion will give a negative test with phloroglucinol reagent.

[1] After administration of urotropine or urotropine derivatives.

Example.—Within 2 days 6 grams of hexamethylene-tetramine were taken. Total urine amounted to 4600 cc. and 200 cc. were taken for the test. Volume of distillate was 1950 cc. For colorimetric determination the distillate was diluted 4 times. Ten cc. of diluted distillate = 2.5 cc. of original distillate gave a reading of 56 = 0.281 mg. of formaldehyde. So the total distillate contained 219.2 mg. of formaldehyde, the amount in 200 cc. of urine. Total urine therefore contained 5.05 grams of formaldehyde. Upon complete hydrolysis 6 grams of hexamethylene-tetramine should give 7.7 grams of formaldehyde, whereas 5.05 grams were found in the urine. This corresponds to 65.4 per cent. of hexamethylene-tetramine taken which was again eliminated in the urine. Thus only 35 mg. of free formaldehyde were found in the acid urine.

Estimation of Formaldehyde in Trioxymethylene Tablets

(Blank and Finkenbeiner[1])

This method is based upon the quantitative oxidation of formaldehyde to formic acid by hydrogen peroxide in a solution rendered strongly alkaline with n-sodium hydroxide solution and titration of the excess of the latter reagent with n-sulphuric acid. Rust[2] employs this method in determining formaldehyde in trioxymethylene tablets:

$$\text{(a)} \quad H.CHO + H_2O_2 = H.COOH + H_2O$$
$$\text{(b)} \quad H.COOH + NaOH = H.COONa + H_2O$$

Procedure.—Add 70 cc. of n-sodium hydroxide solution to 1.9–2 grams of finely ground trioxymethylene tablets in a 250 cc. Erlenmeyer flask. In the course of an hour add 9–10 grams of pure, acid-free 30 per cent. hydrogen peroxide, that is, perhydrol of Merck. At first the latter should be added in small quantity at long intervals to avoid too high temperature and troublesome frothing. Later it may be added in somewhat larger quantity and more rapidly. After about 2 hours set the flask with a funnel on top upon a wire-gauze, warming gently at first but finally to boiling. Add 2 drops of phenol-phthalein solution, then run in n-sulphuric acid in slight excess, titrating back with n-sodium hydroxide solution. One cc. of n-sodium hydroxide, neutralized by formic acid from formaldehyde, corresponds to 0.03 gram of formaldehyde.

Special tests of trioxymethylene tablets should be made for presence of possible alkali and acid.

[1] O. Blank and H. Finkenbeiner: A New Method for the Determination of Formaldehyde. Ber. d. Deutsch. chem. Ges. 31 (1898), 2997.

[2] E. Rüst: Determination of Formaldehyde in Trioxymethylene Tablets. Zeitschr. f. angew. Chemie 19 (1905), 138.

Detection and Estimation of Quinine in Urine

(a) Qualitative Test of Giemsa and Halberkann[1]

This test may be applied to urine direct and is based upon the fact that potassium mercuric iodide precipitates quinine. Following administration of 0.3 gram of quinine hydrochloride, urine will give a positive test.

Preparation of Reagent.—Prepare two solutions, one containing 10 grams of potassium iodide in 50 cc. of water and the other 2.7 grams of mercuric chloride in 150 cc. of hot water. Bring the two solutions together, and add 2.5 grams of glacial acetic acid, thus getting a reagent that keeps.

Even in a dilution of 1:200,000, this reagent will show quinine by a turbidity that disappears on warming (Salomon[2]). Inasmuch as this reagent also precipitates albumin, the following precaution should be taken. If, upon addition of this reagent, the clear, cold, filtered urine remains clear, it contains neither quinine nor albumin. If a milkiness appears and then disappears on warming, quinine is present. If, however, the turbidity persists on warming, albumin is present. If the hot, clear filtrate again becomes turbid on cooling, quinine is also present in the urine in addition to albumin.

Quinine may be detected in urine shortly after the alkaloid has been taken. If quinine is to be identified by fluoresence, or the thalleioquin test, it should first be isolated from urine. For this purpose not too small a quantity of urine (preferably the 24 hour output) is made alkaline with sodium hydroxide solution and shaken with ether. The ether solution is passed through a dry paper, distilled, and the residue tested for quinine. If present, the solution of the residue in dilute sulphuric acid will show blue fluorescence, and a further test of the residue may be made by the thalleioquin, as well as by the erythro-quinine reaction (see pages 214 and 215).

(b) Quantitative Estimation by Meyer

Experiments on man have shown that quinine, taken internally in daily doses of 0.5–1 gram of quinine hydrochloride, is eliminated

[1] G. Giemsa and J. Halberkann: Behavior of Quinine in the Human Organism. Deutsche med. Wochenschr. 1917, No. 48.

[2] H. Salomon: Detection of Quinine in Urine. Ber. d. Deutsch pharm. Ges. 28 (1918), 273.

in large part in the urine unchanged. Schmitz[1] failed to find in urine decomposition-products, especially dihydroxy-quinine described by Kerner. Within 48 hours 26–30 per cent. of the quinine, administered by the mouth in powder-form as the hydrochloride, was again eliminated. During prolonged administration, the daily elimination of quinine in the urine varied between 19 and 35 per cent., on the average about 27 per cent. In subcutaneous administration, combined with urethane, elimination of quinine is less than after internal administration, the daily average being only 16 per cent. That portion of the quinine that does not appear in the urine is destroyed in the organism. Long continued use does not increase the power of the human organism to destroy quinine. Nishi,[2] experimenting upon himself, took by the mouth 0.5 gram of quinine during 72 hours and recovered on the average 34.45 per cent. of the alkaloid in the urine, most of it within 24 hours, the quantity eliminated averaging 25.5 per cent. A large part of the quinine taken is apparently destroyed in the human organism. In his estimation of quinine, Nishi employed the method described by Meyer which makes use of acid quinine citrate, $C_{20}H_{24}N_2O_2.C_6H_8O_7$. This is formed from anhydrous quinine and anhydrous citric acid in ether and crystallizes from hot water in white needles, melting at 240°. On slow heating, it is decomposed at 196–197°, leaving a yellowish brown residue. It has an acid reaction, remains unchanged in air, and is slightly soluble in cold water, 10 cc. of water at room temperature dissolving 0.0182 gram.

Procedure.—Render a measured volume of urine strongly alkaline with sodium hydroxide solution. This is to prevent, during extraction of quinine, transference to the ether of constituents of the urine of the character of resins and pigments. Extract for about 25–30 hours with ether, using an extraction-apparatus provided with electric heating. Then convert quinine to acid quinine citrate by adding an ether solution of citric acid. Animal charcoal should not be used, since it retains quinine. Upon adding bone-charcoal to the ether in the extraction-flask, obtained from a strongly alkaline urine, containing 0.0377 gram of pure quinine, not a trace of alkaloid could be detected in the ether residue.

[1] R. Schmitz: Elimination of Quinine in Human Urine. Arch. f. exper. Path. u. Pharm. 56 (1907) 301.

[2] M. Nishi: A New Method of Estimating Quinine and its Elimination in Urine. Arch. f. exper. Path. u. Pharm. 60 (1909), 312.

Quantitative Estimation of Quinine and Strychnine Together

(Harrison and Gair[1])

Harrison and Gair found the method proposed by Wilson of separating these two alkaloids by means of potassium sulphocyanate entirely useless. The same was equally true of the potassium cyanide method recommended by Flückiger. And finally the ammonium oxalate method of Allen gave quite inaccurate results. On the other hand, these two alkaloids may be separated by means of Rochelle salt. Quinine tartrate, $(C_{20}H_{24}N_2O_2)_2.C_4H_6O_6.2H_2O$, difficultly soluble in water, is precipitated as a white crystalline salt, whereas strychnine tartrate remains in solution.

The necessity of estimating small quantities of strychnine in presence of relatively large quantities of quinine may arise in the examination of pharmaceutical preparations and specialties, such as Compound Syrup of Hypophosphites and Easton's Syrup.

Procedure.—Render the solution of mixed alkaloids, containing about 0.05–0.1 gram of strychnine, in about 40 cc. of water faintly acid with sulphuric acid. Add enough ammonia to cause slight turbidity, then 15 grams of solid Rochelle salt and more ammonia, leaving the liquid acid to litmus paper. Heat for 15 minutes upon the water-bath and set aside for 2 hours until entirely cold. Filter precipitated quinine tartrate by suction and wash with aqueous Rochelle salt solution (15 grams of salt in 45 cc. of water), containing 1–2 drops of dilute sulphuric acid. To determine strychnine, add sodium hydroxide solution to the combined filtrate and wash-water from quinine tartrate until the reaction is alkaline. Extract 3–4 times with chloroform, pour the chloroform extract through a dry filter and distil in a weighed flask to about 4 cc. Add 10 cc. of absolute alcohol and evaporate to dryness upon the water-bath. To remove quinine adhering to strychnine, extract the dry residue 2–3 times with 1 cc. portions of ether,[2] dry at 100° and weigh. This residue consists of pure, quinine-free strychnine.

[1] E. F. Harrison and D. Gair: Quantitative Separation of Strychnine and Quinine. Pharmac. Journ. (4) 17 (1903), 165.

[2] More ether than prescribed should not be taken, for otherwise weighable quantities of strychnine are dissolved.

Quantitative Estimation of Strychnine in Presence of Quinine

(Simmonds[1])

This method depends upon the fact that potassium ferrocyanide in slight excess will precipitate strychnine from acid solution. Under these conditions quinine remains in solution and is not precipitated until a larger excess of reagent is used. For the purpose of getting strychnine free from quinine precipitated at the same time, the mixed alkaloids first obtained are subjected to reprecipitation. Simmonds applied this method to the examination of Easton's Syrup, 50 cc. of which are said to contain 0.5442 gram of quinine and 0.0285 gram of strychnine. There is no reason why it should not be used for other mixtures of these two alkaloids.

Procedure.—First add citric acid, then excess of ammonia to 50 cc. of the aqueous solution (Easton's Syrup, for example), and extract with chloroform-ether mixture. Distil the chloroform-ether extract in a tared flask, weigh the residue dried to constant weight in a drying-closet, or better in a vacuum-desiccator (Fig. 13, page 96), and dissolve in 50 cc. of 10 per cent. sulphuric acid. Then add 5 cc. of 4 per cent. potassium ferrocyanide solution, after several hours filter the precipitate, and wash 3 times with 5 cc. portions of 10 per cent. sulphuric acid. Wash the precipitate into a rather small separatory funnel with 10 cc. of 10 per cent. ammonia and some water, and extract 3 times, using 15, 10 and finally 5 cc. of chloroform. From this chloroform solution extract strychnine, which contains a little quinine, three times, using 30, 20 and 10 cc. portions of 20 per cent. sulphuric acid. Repeat precipitation with potassium ferrocyanide, as well as the entire process, finally evaporate the chloroform solution, adding alcohol toward the end to prevent spattering, and weigh the residual strychnine, now quinine-free, after drying for an hour at 100°.

Examination of Chloral Urine

Detection of Urochloralic Acid

Only traces of chloral hydrate, taken into the body by the mouth, reach the urine as such. The greater part is eliminated in the urine as urochloralic acid, a conjugated glycuronic acid. Consequently after poisoning from chloral hydrate urine should be tested for urochloralic acid. Such "chloral urine" behaves like a sugar urine in reducing Fehling's solution but differs in exhibiting distinct laevorotatory properties (v. Mering and Musculus[2]).

(a) **Isolation Method of v. Mering.**[3]—Evaporate the urine upon the water-bath to a syrupy consistency, strongly acidify with dilute sulphuric acid, and thoroughly extract several times with a mixture of 3 volumes of ether and 1 volume of alcohol. Distil alcohol and ether as completely as possible from these

[1] C. Simmonds: Estimation of Strychnine in Presence of Quinine. The Analyst 39 (1914), 81.

[2] v. Mering and Musculus: A New Body in Chloral Urine. Ber. d. Deutsch. chem. Ges. 8 (1875), 662.

[3] v. Mering: Behavior of Chloral Hydrate and Butyl-chloral Hydrate in the Organism. Zeitschr. f. physiol. Chem. 6 (1882), 480

extracts, exactly neutralize the residue with potassium carbonate or hydroxide, and evaporate. Take up the residue in 90 per cent. alcohol, filter, precipitate the filtrate with ether, wash the precipitate repeatedly with ether and absolute alcohol, and dry over sulphuric acid. Then boil the precipitate with absolute alcohol and filter the alcoholic solution hot. If the urine contains urochloralic acid, this solution as it cools will deposit the potassium salt of this acid in clusters of colorless, silky needles. This potassium salt should be dried over sulphuric acid and washed several times with absolute alcohol and ether to remove adherent impurities that may still be present. Free urochloralic acid may be obtained from this potassium salt by dissolving in a little water, adding dilute hydrochloric acid, and thoroughly extracting with a mixture consisting of 8 volumes of ether and 1 volume of alcohol. Distil the ether-alcohol solution and add moist silver oxide to the residue until silver chloride ceases to separate. By means of hydrogen sulphide quickly remove silver from the filtrate, filter and carefully evaporate the filtrate to a thin, syrupy consistency. In the course of a few hours, free urochloralic acid will crystallize in clusters of colorless, silky, star-shaped needles.

(b) **Isolation Method of E. and R. Külz.**[1]—This method also prescribes preliminary evaporation of urine upon the water-bath to a thick syrup, strong acidification with dilute sulphuric acid, and several thorough extractions with an alcohol-ether mixture. These extracts are freed from alcohol and ether by distillation. The residue is first precipitated with neutral lead acetate and the filtrate from this precipitate is then precipitated with basic lead acetate. The latter precipitate contains lead urochloralate. By means of hydrogen sulphide it is decomposed, lead sulphide removed by filtration, and hydrogen sulphide expelled by warming the filtrate. The latter is neutralized with barium hydroxide solution, concentrated by evaporation upon the water-bath, and freed from barium by careful addition of the requisite quantity of dilute sulphuric acid. The filtrate from barium sulphate is evaporated by gentle heat to a syrup, and dried in vacuum-desiccator over sulphuric acid. The dry residue is extracted with ether and the filtered ether extract upon evaporation deposits urochloralic acid in crystals.

Urochloralic Acid, $C_8H_{11}Cl_3O_7$.—This compound appears as colorless, silky needles melting at 142°. It is readily soluble in water and alcohol; soluble in a mixture of alcohol and ether; and insoluble in pure ether. On boiling it reduces alkaline copper, silver and bismuth solutions and colors yellow an indigo solution rendered faintly alkaline with sodium carbonate. It is precipitated by basic but not by neutral lead aceate. On boiling with potassium hydroxide solution it turns brown, then evolves a caramel odor, and gives up chlorine to potassium. The specific rotation of potassium urochloralate for yellow light is $[\alpha]_D = -60°$. Heated under a reflux for 2–3 hours with 7 per cent. hydrochloric or sulphuric acid, urochloralic acid is hydrolyzed into trichlor-ethyl alcohol and glycuronic acid:

[1] E. Külz: Fate of Chloral Hydrate and Butyl-chloral Hydrate in the Animal Body. Arch. f. d. ges. Physiol. 28 (1883), 506; R. Külz: Formation and contribution to the Knowledge of Urochloralic Acid and the Chlorine Cleavage-products of Urochloralic Acid and Urobutylchloralic Acid. Arch. f. d. ges. Physiol. 33 (1884), 227.

$$C_8H_{11}Cl_3O_7 + H_2O = CCl_3.CH_2.OH + HOC.(CHOH)_4.COOH$$

Urochloralic acid — Trichlor-ethyl alcohol — Glycuronic acid

Urochloralic acid possibly has the following structural formula:

$$CCl_3.CH_2.O.CH.CH(OH).CH(OH).CH.CH(OH).COOH$$
$$\quad\quad\quad

cc. of water, until 5 cc. of distillate give a negative test with Millon's reagent. Add to the total distillate in a capacious flask excess of calcium carbonate. Pass in a current of pure carbon dioxide and distil again. Continue distillation until the test with Millon's reagent is negative. Determine volatile phenols, finally obtained in the distillate as a result of this procedure, by the Kossler-Penny method.

For this purpose render the distillate strongly alkaline with an accurately measured volume of 0.1 n-sodium hydroxide solution (20–30 cc. are usually sufficient), warm by immersing the flask in water at 60°, and at once treat with 10–15 cc. more of 0.1 n-iodine solution than of the alkaline solution. When cold rinse sublimed iodine from the walls of the flask with the solution, acidify with dilute hydrochloric acid, and titrate excess iodine with 0.1 n-thiosulphate solution, adding starch solution as indicator toward the end. The liquid holds iodinated phenols as a red precipitate, having a violet appearance on account of iodo-starch. The change of color from violet to red, as the last trace of iodine is removed, may however be distinctly recognized. According to the equation above, 6 atoms of iodine combine with 1 molecule of phenol; therefore 1000 cc. of 0.1 n-iodine solution correspond to 1/60 gram of $C_6H_5.OH$ = 94.05:60 = 1.5675 gram of phenol.

(b) **Method of Ellinger-Hensel.**[1]—This is an extraction method in which the first distillate obtained by Mooser's method from urine acidified with phosphoric acid is repeatedly shaken with large quantities of ether. The ether extract is shaken with sodium carbonate solution to remove volatile acids and then with dilute sodium hydroxide solution when the phenols pass into solution as phenolates. Most of the free alkali is neutralized by sulphuric acid and phenols are determined by the Kossler-Penny method.

Procedure.—Render 500 cc. of urine faintly alkaline with sodium hydroxide solution and evaporate upon the water-bath to about 100 cc. Transfer to a capacious distilling flask, rinsing the dish with water and having a total volume of 150–200 cc. Add 25 cc. of approximately 85 per cent. phosphoric acid, arrange for distillation, and distil to 100 cc. Add 50 cc. of water to the distillation flask and distil again. Repeat this operation until the distillate gives a negative test with Millon's reagent. The number of such distillations will vary from 6–20. Up to this point the method is exactly like that of Mooser. Make four extractions of distillate, using each time one-fourth the volume of ether that has previously been purified by shaking five times with dilute sodium hydroxide solution. The total ether extract is first well shaken four times with 4 per cent. sodium carbonate solution and finally four times with approximately n-sodium hydroxide solution. Excess of sodium hydroxide in the aqueous, alkaline solution of phenolate should be neutralized until the alkalinity corresponds to 20 cc. of 0.1 n-sodium hydroxide. If, for example, 250 cc. of normal sodium hydroxide were used, 248 cc. of normal sulphuric acid should be added. Determine phenols in this alkaline liquid by the method of Kossler and Penny.

Notes.—This method, owing to omission of the second distillation, that is, the one over calcium carbonate in a stream of carbon dioxide, is somewhat more rapid than that of Mooser but requires more manual skill than the latter. This extrac-

[1] A. Ellinger and M. Hensel: Procedure for Determination of Phenol in Urine. Zeitschr. f. physiol. Chem. 78 (1912), 372.

tion method of estimating phenols in urine also gives thoroughly reliable results with urines rich in carbohydrates. The method of Mooser also gives accurate results so that the two methods are much alike.

2. Estimation in Urine of Total Sulphate-Sulphuric and Ethereal Sulphuric Acid

Sulphuric acid occurs in human urine in two forms. So-called sulphate sulphuric acid is capable of yielding SO_4''-ions, as from sulphates. In union with phenol, p-cresol and indoxyl, it forms aromatic ethereal sulphuric acid. Sulphate-sulphuric acid is also designated as A-sulphuric acid, and ethereal sulphuric acid as B-sulphuric or conjugated sulphuric acid. Therefore total sulphuric acid of urine consists of sulphate- and ethereal-sulphuric acid. The 24 hour urine of a healthy, adult man on a mixed diet contains 1.5–3.0 grams of total sulphuric acid, expressed as SO_3. Its quantity depends chiefly upon the amount of proteins taken with the food and assimilated. In a healthy man on a mixed diet ethereal sulphuric acid amounts to about one-tenth of the total sulphuric acid. Consequently under normal nutrition conditions the ratio of A- to B-sulphuric acid is about 10:1. But the quantity of ethereal sulphuric acid is subject to considerable variation and is dependent upon: 1. Character of food; 2. Intensity of intestinal putrefaction; 3. Direct introduction of those aromatic substances that combine in the human organism with sulphuric acid to form ethereal sulphuric acid. In poisoning from carbolic acid, cresols, lysol, thymol, in brief by any phenol whatever, ethereal sulphuric acid usually shows a marked increase at the expense of sulphate-sulphuric acid, so that all but traces of the latter may disappear. As stated above, the amount of ethereal sulphuric acid normally present in urine depends principally upon the degree of protein-putrefaction taking place in the intestines. The more intense this is, the more ethereal sulphuric acid will be present. Following thorough disinfection or complete evacuation of the intestines, for example, after administration of calomel, conjugated sulphuric acid may be greatly diminished in quantity or entirely disappear from urine.

(a) Total Sulphuric Acid

Since conjugated sulphuric acid differs from sulphate-sulphuric acid in not being precipitated by soluble barium salts, it should first be warmed with hydrochloric acid and hydrolyzed into its constituent parts:

$$C_6H_5O.SO_2.OK + H_2O = C_6H_5.OH + KHSO_4$$

if total sulphuric acid in urine is to be estimated.

Procedure.—Dilute 50 cc. of previously filtered urine with twice its volume of water. Add 10–15 cc. of hydrochloric acid (20 per cent. HCl), heat nearly to boiling, and precipitate with a slight excess of barium chloride solution, the concentration of which is not more than 10 per cent. Allow the mixture to stand for 3–4 hours upon a hot water-bath and in the cold until the following day, to permit complete precipitation of barium sulphate that is at first held in solution by hot hydrochloric acid. Then decant the clear, supernatant liquid through a small, ash-free filter. Stir the precipitate in the beaker with a little hot water, allow to settle, and again decant the supernatant liquid through the same filter. Repeat this operation until the filtrate fails to give tests for barium and chloride with sulphuric acid and silver nitrate. Finally transfer the precipitate without loss to

the same filter. Mixed with it are brown, resinous substances which for the most part may be brought into solution by treatment with hot alcohol according to the procedure of Baumann. Separate the dried precipitate as completely as possible from paper and burn the latter on a platinum spiral. Then ignite filter-ash and precipitate to constant weight in a weighed platinum or porcelain crucible. By weight 100 parts of barium sulphate correspond to 34.29 parts by weight of SO_3, or 41.14 of SO_4, or 42.01 of H_2SO_4.

Notes.—Obviously the precipitate of barium sulphate may also be collected, as prescribed by Folin, in a weighed Gooch crucible with asbestos mat and washed with cold water.

(b) Ethereal or Conjugated Sulphuric Acid

1. Method of Baumann.[1]—This estimation is based upon the observation of Baumann that conjugated sulphuric acids in urine are not decomposed when warmed for 1 hour with dilute acetic acid. But they are completely hydrolyzed, if warmed for a few minutes, or allowed to stand for a few hours at ordinary temperature, with only a small quantity of hydrochloric acid. For this reason sulphuric acid in urine as simple sulphates should be precipitated only in acetic acid solution.

Procedure.—Add to 50 cc. of urine the same volume of water, 5 cc. of dilute acetic acid, and excess of barium chloride solution. Warm upon the water-bath until the precipitate has settled clear which usually requires about 30–45 minutes. Wash the precipitate upon the filter first with water, then with cold, dilute hydrochloric acid,[2] and finally with water. The weight of dried and ignited precipitate is barium sulphate corresponding to sulphate-sulphuric acid in the urine.

Combine filtrate and wash-water, add some dilute hydrochloric acid, and warm until the newly formed precipitate of barium sulphate has settled clear. This requires from 1–2 hours. Heating for this length of time insures complete hydrolysis of all conjugated sulphuric acids. Usually the precipitate of barium sulphate is mixed with brown, resinous matter which may be removed for the most part by washing the precipitate upon the filter with hot alcohol. Finally wash the precipitate thoroughly with hot water. The weight of this second precipitate of barium sulphate corresponds to the quantity of conjugated sulphuric acid in the urine.

To avoid the tedious operation of washing the first barium sulphate precipitate, rinse the mixture of urine and barium chloride acidified with acetic acid together with the precipitate into a graduate. Bring to a definite volume with water, shake thoroughly, allow to settle, and filter a definite volume through dry paper for use in estimating conjugated sulphuric acid. This is always the method of procedure, when only conjugated sulphuric acid is to be estimated. In that case take 100 cc. of urine, dilute after addition of acetic acid (about 5 cc.) with about 60 cc. of water, and finally rinse the entire mixture without loss into a 200 cc. graduated flask, bringing the liquid to the mark with water. Conjugated sulphuric acid may then be estimated in 100 cc. of filtrate, corresponding to 50 cc. of urine.

[1] E. Baumann: Estimation of Sulphuric Acid in Urine. Zeitschr. f. physiol. Chem. 1 (1877–78), 70.

[2] For the purpose of dissolving barium phosphate mixed with barium sulphate.

2. **Method of Salkowski.**[1]—The acetic acid liquid resulting from Baumann's procedure is frequently difficult to filter and barium sulphate often passes through the paper. Salkowski overcomes these difficulties by modifying the procedure somewhat. Precipitate 100 cc. of urine with an equal volume of baryta mixture, consisting of two volumes of cold, saturated barium hydroxide solution and one volume of cold, saturated barium chloride solution. Filter, render 100 cc. of filtrate (50 cc. of urine) strongly acid with hydrochloric acid, heat nearly to boiling, and digest upon the water-bath for 1–2 hours. In other respects the method is the same as that described above.

(c) Sulphate-sulphuric Acid

1. Sulphate-sulphuric acid is estimated by Baumann's method given under 2a (see page 559).

The barium precipitate, due to sulphate-sulphuric acid and obtained from urine acidified with acetic acid, is always more or less impure from barium phosphate. In all cases therefore it should be purified as described by treatment in the cold with dilute hydrochloric acid which dissolves barium phosphate.

2. **Estimation by Difference.**—Estimate total sulphuric acid in a test-portion of urine (50 cc.) and at the same time conjugated sulphuric acid in the same volume of urine, preferably by the method of Salkowski. The difference in weight of these two precipitates corresponds to the quantity of sulphate-sulphuric acid in the urine.

DETECTION OF MORPHINE IN ORGANS

Methods of Cloetta and Marquis

1. **Method of Cloetta.**[2]—First pass the given organ, or organs, through a meat-grinder, add water and triturate in a mortar. Then acidify with acetic acid, heat to boiling and filter. Wash out paper and precipitate several times with hot water. Add basic lead acetate to the filtrate and wash the precipitate with hot alcohol until the test with Froehde's reagent is negative. Free the filtrate from lead with hydrogen sulphide and expel the latter by air. Again filter and evaporate the filtrate to 20 cc., keeping it always acid to acetic acid. Render alkaline with ammonia and thoroughly extract 4–6 times with isobutyl alcohol. Separate the isobutyl alcohol extract and filter after 24 hours. Evaporate this filtrate at as low a temperature as possible which requires about 10 hours. Dissolve the residue in a mixture of absolute alcohol, chloroform and benzene (2:2:1 volume), filter the solution after 24 hours, and again evaporate. Dissolve the residue in water containing acetic acid, filter and

[1] E. Salkowski: Virchow's Archiv. 79, 552.

[2] M. Cloetta: Behavior of Morphine in the Organism and Causes of Habituation. Arch. f. exper. Path. u. Pharm. 50 (1903), 453.

evaporate to 2 cc. Add a drop of strong ammonia, making certain that the reaction is alkaline. Morphine separates at once, or after seeding the solution with a crystal of free morphine base. To determine morphine quantitatively, collect the precipitate upon a weighed filter, wash with not more than 2 cc. of water, and weigh after drying in a vacuum desiccator or at 100°.

2. Method of Marquis.[1]—Finely comminute the given organs and stir with water until the mass is thin. Acidify faintly with hydrochloric acid and warm for a short time, not longer than 5 minutes, upon a boiling water-bath in order to bring about cleavage of morphine that has entered into combination. Then nearly neutralize with ammonia and strain quickly. Stir the residual material twice with hot water and strain each time. Evaporate the combined filtrates upon the water-bath. Stir the residue with alcohol until uniform, pour through paper moistened with alcohol, and wash several times with this solvent. Evaporate the combined alcoholic filtrates at about 50°, extract the residue with water, filter and again evaporate. Again purify this residue in the same manner by dissolving in alcohol and then in water. Acidify the aqueous solution with hydrochloric acid and remove foreign matter by extracting first with cold and then with slightly warm ether. Render this purified aqueous solution of morphine alkaline with sodium bicarbonate and extract warm several times with acetic ether as well as with isobutyl alcohol. If the residue remaining after evaporation of the acetic ether-isobutyl alcohol extracts shows color, it should be further purified. For this purpose dissolve the residue in hot amyl alcohol and thoroughly extract this solution with hot water containing a little dilute sulphuric acid. Morphine will pass into the acidulated water, whereas colored impurities for the most part will remain in the amyl alcohol. Render the sulphuric acid solution faintly alkaline with ammonia and repeatedly extract with chloroform containing alcohol (1 part of alcohol to 9 parts of chloroform). The residue will consist of practically pure and frequently crystalline morphine. Finally this morphine residue may be dissolved in the smallest possible quantity of hot chloroform and mixed with 50 times the volume of petroleum ether. If this solution is cooled with ice and allowed to stand for 24 hours, usually it will deposit morphine in crystalline condition.

[1] E. Marquis: On Morphine remaining in the Human Organism. Dissertation, Dorpat 1896.

COLORIMETRIC ESTIMATION OF MORPHINE IN URINE

Applicable to Examination of Urine of Persons Poisoned by Opium or Morphine and of Morphinists

Of all the color-reactions of morphine the most suitable for this purpose are those due to formation of azo-dyestuffs when diazotized primary, aromatic amines are coupled with this alkaloid. Such reactions are possible because of the phenolic character of morphine. Diazo-benzene sulphonic acid, for example, couples readily with morphine, forming a beautiful red dyestuff.

Lautenschläger[1] uses diazo-benzene sulphonic acid as prepared, whereas Autenrieth and Quantmeyer[2] prefer freshly prepared diazotized sulphanilic acid from which all free nitrous acid, that may cause interference, is removed by means of urea:

$$CO(NH_2)_2 + 2HONO = CO_2 + 2N_2 + 3H_2O$$

Coupling for dyestuff-formation is first conducted in acid solution. Then addition of sodium hydroxide solution produces an orange- to blood-red dye solution, the color-strength of which is determined in the Autenrieth-Koenigsberger colorimeter by means of a calibrated comparison-wedge. Other opium alkaloids and also quinine, codeine, caffeine and strychine cause no interference with this color-reaction.

Requisites.—1. Sulphanilic acid solution prepared by dissolving 2 grams of sulphanilic acid in 100 cc. of approximately 7 per cent. hydrochloric acid. 2. Sodium nitrite solution (1 per cent. aqueous solution). 3. Concentrated sodium hydroxide solution prepared from purest sodium hydroxide. 4. Starch-potassium iodide paper. 5. Saturated aqueous solution of urea (about 50 per cent.). 6. Morphine hydrochloride solution of known strength.

Calibration of Comparison-wedge.—To calibrate the comparison-wedge for the construction of the calibration-curve shown below, a solution of purest morphine hydrochloride, $C_{17}H_{19}NO_3.HCl.3H_2O$, rendered faintly acid with hydrochloric acid, was used. In 100 cc. of this solution were 98.5 mg. of morphine hydrochloride. So each cc. had a free morphine-content of 0.75 mg., since morphine is $C_{17}H_{19}NO_3$ (Mol. Wt. 285).

Procedure.—In each case introduce 1 cc. of sulphanilic acid solution (1) into a 10 cc. graduate set in ice-water to keep well cooled and add 5 drops of sodium nitrite solution (2). After thorough shaking, discharge excess of nitrous acid by adding 1 cc. or sufficient urea solution (5) to produce a negative test with starch-iodide paper. Now add from a capillary pipette varying but carefully measured quantities, that is, 1–5 cc., of morphine hydrochloride solution (6), shake for 3 minutes and render strongly alkaline with 10 drops of sodium hydroxide solution (3). Immediately a deep red color appears. Bring the volume of liquid to the

[1] L. Lautenschläger: The Diazo Reaction of Morphine. Archiv. d. Pharmaz. 257 (1919), 13.

[2] From the unpublished manuscript of the authors.

10 cc. mark with water, shake, pour the mixture into a dry test-tube, and place for about 5 minutes in a water-bath warmed to 50°. Under these conditions the red color is developed to a maximum. Cool this solution and pour into the glass trough of the colorimeter. Adjust the comparison-wedge to the point where the color is the same as that of the solution in the trough and record the reading. In the calibration of the comparison-wedge the following values were obtained:

Morphine solution in cc.....	1	2	3	4	5
Morphine in 10 cc. in mg.....	0.75	1.5	2.25	3.0	3.75
Morphine in 1 cc. in mg.....	0.075	0.15	0.225	0.3	0.375
Scale-reading.....	71	58	46	34	22

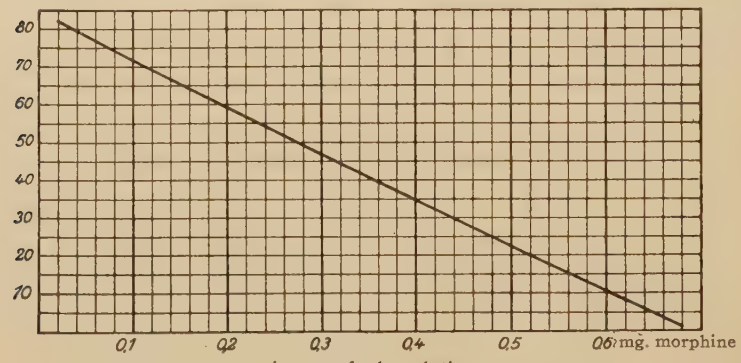


FIG. 56.—Morphine in 1 cc. of color solution.

The calibration-curve is constructed by recording in each case the quantities of morphine in mg. upon the abscissae and the readings for equal color-strength upon the ordinates of coördinate paper. The line connecting the intersecting points of the two coördinates is the calibration-curve.

On this curve a difference of 1 mm. corresponds to ±0.002 mg. of morphine. By means of this comparison-wedge quantities of 0.05–0.5 mg. of morphine, dissolved in each case in 1 cc. of color solution, may be estimated colorimetrically.

It is worthy of note that morphine in coupling with diazonium compounds loses its physiological action (Lautencshläger).

Estimation of Morphine in Urine

For this purpose take rather a large quantity of urine, if possible, the 24 hour output. Acidify the urine strongly with tartaric acid and evaporate to a syrup upon the water-bath. Stir with 4–5 times the quantity of alcohol, filter and again evaporate the filtrate upon the water-bath, or distil the alcohol upon the water-bath. Dissolve the residue in 30–40 cc. of water (if the reaction is not acid, add more tartaric acid) and thoroughly extract the solution with ether, in order to remove as completely as possible coloring-matter and other

substances that cause more or less interference. Then render the solution alkaline with ammonia, or sodium bicarbonate, and at once extract thoroughly with a boiling mixture of 1 part of absolute alcohol and 9 parts of chloroform. If this causes an emulsion, heat upon the water-bath in a flask and shake. Usually this will bring about an immediate separation into two liquid layers. The alcohol-chloroform extract should be dried in a flask with anhydrous sodium sulphate. In the same manner several extractions of the aqueous liquid should be made in a separating funnel with boiling alcohol-chloroform mixture. This operation must be continued until the residue obtained by evaporating a test-portion of the extract fails to give a positive result by the Marquis morphine test[1] (see page 231). Allow the united alcohol-chloroform extracts, after gentle warming upon the water-bath, to stand until entirely clear. Then pour through dry paper into a weighed Erlenmeyer flask and distil as nearly as possible to dryness. The best procedure is to dry to constant weight in a vacuum desiccator. This gives the quantity of crude morphine in the urine. A colorimetric estimation of this crude morphine is made by dissolving it in a little dilute acetic acid and diluting the solution with water, depending upon the weight of morphine, to 50, 100 or more cc., so that a volume of 1–5 cc. of this solution is within the reading-range of the calibrated comparison-wedge of the colorimeter. If the quantity of solution of morphine in acetic acid is sufficient, determinations of morphine in the manner described should always be made with different volumes (1, 2, 3 cc.).

Example.—The weight of crude morphine obtained from the 24 hour urine (1800 cc.) of a morphinist was 205 mg. The solution in dilute acetic acid was diluted with water to 100 cc. In accordance with the above directions 10 cc. of color solution were prepared from 1 cc. of this solution. This gave a reading of 54 = 1.8 mg. of free morphine in 10 cc. of the color solution (see calibration curve), or $100 \times 1.8 = 180$ mg. of morphine in the entire solution representing the 24 hour urine. Differences between the gravimetric and colorimetric estimations are to be traced to impurities in the crude morphine. It is recommended that a gravimetric and colorimetric estimation of morphine always be made.

Notes.—The great advantage of the colorimetric as compared with the gravimetric method is that it permits estimation of very small quantities of morphine.

[1] In an unpublished paper Autenrieth and Quantmeyer have shown as a result of the examination of the urine of morphinists that in every instance six extractions with boiling alcohol-chloroform mixture (1:9) completely removed morphine, whereas when pure chloroform was used even ten extractions were not sufficient to produce this result.

In many cases even 100 or 50 cc. of urine of a morphinist are sufficient for a single morphine determination.

EXAMINATION OF PYRAMIDONE URINE[1]

Detection of Antipyryl-urea and Rubazonic Acid

To isolate rubazonic acid, present in urine to the extent of only about 3 per cent. of the pyramidone taken, acidify freshly collected urine with hydrochloric acid and allow to stand in an open vessel. Crude rubazonic acid separates in red flocks. Filter the crude product, add ammonia, and extract repeatedly with acetic ether. The dyestuff is dissolved by acetic ether as an ammonium compound having a purple color. Upon distilling the solvent, rubazonic acid is obtained in splendid, red needles free from ammonia. If recrystallized from hot acetic ether or glacial acetic acid, it may be obtained pure. Rubazonic acid melts at 184°. It is insoluble in water and dilute acids; very difficultly soluble even in hot alcohol, and more easily soluble in acetic ether, chloroform, benzene, acetone and glacial acetic acid. Alkalies dissolve it readily producing a purple color. This color is discharged by reducing agents, such as ammonium sulphide or glacial acetic acid and zinc dust. In air, however, the leuco-compound is soon oxidized and the pigment reappears.

Antipyryl-urea.—To isolate antipyryl-urea, render the hydrochloric acid filtrate from rubazonic acid faintly alkaline with sodium carbonate, and evaporate at gentle heat to a thin syrup. Decant from sodium chloride that crystallizes and mix while still warm with four times its volume of a mixture of equal volumes of alcohol and ether. Stir frequently and allow to stand for several days. Decant the solution, treat the residue with five volumes of alcohol-ether mixture, and repeat this procedure once. In this manner a dark brown aqueous syrup (A) interspersed with crystals is obtained and also a highly colored alcohol-ether solution (B). After decolorization with animal charcoal, the former exhibits strong laevo-rotatory power and is found to contain a conjugated glycuronic acid. Distil the solvent from solution (B), dissolve in considerable water the residual crystalline mass consisting chiefly of urea, and acidify with dilute sulphuric acid until the acidity is 5 per cent. Precipitate with phospho-tungstic acid, carefully wash the precipitate with 5

[1] M. Jaffé: The Red Pigment appearing in Urine following Use of Pyramidone. Ber. d. Deutsch. chem. Ges. 34 (1901), 2737; Antipyryl-urea, a Metabolic Product of Pyramidone. Ber. d. Deutsch. chem. Ges. 35 (1902), 2891.

per cent. sulphuric acid, decompose by gently warming with barium hydroxide, and remove excess of barium from the filtrate with carbon dioxide. Evaporate the filtrate from barium carbonate to a thin syrup, best *in vacuo*. It then solidifies to a colorless, crystalline mass, consisting of two substances one is which gives a violet color with ferric chloride, whereas the other does not. They may be separated by treatment of the aqueous solution with considerable acetone, whereupon the compound that does not react with ferric chloride is precipitated. It consists of creatine and is formed as an artificial product from creatinine in urine. The substance not precipitated by acetone, when freed from adherent creatine by recrystallization from water, consists of antipyryl-urea, or uramido-antipyrine. The aqueous solution of this uramido-compound gives a violet color with ferric chloride. When warmed with Millon's reagent, it becomes first yellow, red on longer heating, and finally deposits a red precipitate. It melts at 247–248°. This substance is rather easily soluble in water, more difficultly soluble in alcohol and insoluble in ether.

DETECTION AND ESTIMATION OF VERONAL IN VERONAL URINE

Veronal, according to Fischer and v. Mering,[1] in large part is eliminated unchanged by the kidneys and then 60–70 per cent. and more appears in the urine. Following administration of 4 grams of veronal during 2 days, 2.49 grams 62 per cent. were recovered from 5 days urine. The method used, however, was not absolutely quantitative, nor was elimination of veronal entirely complete after 5 days. Therefore in suspected poisoning by veronal or medinal, urine should first be examined. Fischer and v. Mering evaporated a considerable quantity of urine under diminished pressure to about one-fifth its original volume and extracted with a large volume of ether, since veronal is not very soluble in this solvent. Ether was distilled and the residue, usually highly colored, was dissolved in as little boiling water as possible. This solution was then boiled for about 15 minutes with animal charcoal and filtered hot. The nearly colorless filtrate, when cooled with ice, deposited practically pure veronal.

[1] E. Fischer and J. v. Mering: On Veronal. Die Therapie der Gegenwart (= Modern Therapy) 45 (1904).

Molle and Kleist[1] first add lead acetate solution to urine as long as it causes a precipitate, filter, remove lead from the filtrate by hydrogen sulphide and filter from lead sulphide. Hydrogen sulphide is expelled by heat and the urine, after dilution with twice its volume of water, is boiled with animal charcoal. The filtrate, after concentration upon the water-bath to smaller volume, is cooled, saturated with sodium chloride and repeatedly extracted with ether. The ether solution filtered through dry paper leaves upon distillation nearly pure veronal.

By the following procedure Autenrieth[2] detected veronal in urine and made approximately quantitative determinations. Render 500 cc. or more of urine strongly acid with tartaric acid and evaporate in a porcelain dish upon a boiling water-bath as rapidly as possible to a thin syrup. Stir with 100–150 cc. of alcohol, filter and again evaporate upon a water-bath. Dissolve the residue in about 60 cc. of water and thoroughly extract 2–3 times with 50 cc. portions of ether. Allow the combined ether extracts to settle for a short time in a flask, pour the ether through a dry filter and distil. If veronal is present, the residue usually solidifies to a crystalline mass. Veronal to some extent separates in large brownish crystals impure from pigment, oxalic, hippuric and tartaric acids. The cold residue may be purified by dissolving cold in excess of sodium carbonate solution (1:10) and allowing to stand about 1 hour with animal charcoal. Filter and thoroughly extract veronal from the filtrate, after acidification with dilute sulphuric acid, 2–3 times with 50 cc. portions of ether. To determine veronal quantitatively, distil the combined ether extracts in a dry weighed flask, dry the solid residue at gentle heat (50–60°) and weigh. The flask may be most satisfactorily dried in a large vacuum-desiccator, equipped with 3–4 flat dishes containing a little concentrated sulphuric acid and placed one above the other (Fig. 13, page 96). By use of this apparatus the weight is constant after a few hours. Veronal thus obtained usually has little color and is sufficiently pure for the following tests:

1. When heated cautiously, it easily sublimes.

2. When treated with sodium, potassium or ammonium hydroxides, as well as with alkaline carbonate solutions, it dissolves and is reprecipitated when these solutions are acidified.

[1] B. Molle and H. Kleist: On Veronal. Arch. d. Pharmaz. 242 (1904), 401.

[2] W. Autenrieth: A Case of Poisoning by Medinal and the Detection of Veronal in Urine in such Poisoning. Ber. d. Deutsch. pharm. Ges. 31 (1921), H. 3.

3. A white, gelatinous precipitate, soluble in a large excess of reagent, is given by Millon's reagent, or by a solution of 1 gram of yellow mercuric oxide in 2 cc. of nitric acid. Denigès[1] reagent may also be used.

4. When warmed with sodium hydroxide solution, it gives off vapors that turn litmus blue.

5. If the quantity of veronal permits and is sufficiently pure, percentage of nitrogen may be determined by the Kjeldahl or Dumas method.

Following this procedure, the author recovered 0.502 gram of veronal when 0.548 gram was added to 500 cc. of urine. Veronal thus obtained is not entirely pure and usually has too low a melting-point. If this determination is to be made, veronal should be recrystallized from boiling water with addition of animal charcoal, or dissolved in cold sodium carbonate solution, decolorized with animal charcoal, filtered and reprecipitated with dilute hydrochloric or sulphuric acid. If veronal is not absolutely pure, it melts at 187–188°.

DETECTION OF SALICYLIC ACID IN FOODS AND BEVERAGES

1. In Wine

(German Official Directions for Chemical Examination of Wine)

Put 50 cc. of wine in a cylindrical separating funnel with 50 cc. of a mixture of equal parts of ether and petroleum ether. Shake frequently, taking care not to form an emulsion but yet to mix the liquids thoroughly. Remove the ether-petroleum ether layer, pour through a dry filter, evaporate upon the water-bath, and add a few drops of ferric chloride solution to the residue which becomes red-violet if salicylic acid is present. But if the color is black or dark brown, add a few drops of hydrochloric acid, dissolve in water, extract with ether-petroleum ether and proceed with the extract as just described.

2. In Milk[2]

Mix 100 cc. of milk with 100 cc. of water at 60°. Precipitate with acetic acid and mercuric nitrate solution, using 8 drops of each, shake and filter. Extract the filtrate with 50 cc. of ether, evaporate the ether, dissolve the residue in 5 cc. of hot water and test the filtered solution for salicylic acid with dilute ferric chloride solution (sp. gr. 1.005–1.010).

[1] See "The Preparation of Reagents" page 641.

[2] Method of Ch. Girard: Zeitschr. f. analyt. Chem. 22 (1883), 277.

3. Official Directions for Detection of Salicylic Acid and Compounds in Meat and Meat Products

(Methods of Analysis of German Meat Inspection Act)

Mix 50 grams of finely comminuted meat in a beaker with 50 cc. of 2 per cent. sodium carbonate solution until uniform and allow to soak cold for 30 minutes. Then cover the beaker with a clock-glass, set in a boiling water-bath for 30 minutes, and stir occasionally. Transfer the contents of beaker while still warm to a piece of cheese-cloth and press. Add 5 grams of sodium chloride to the expressed liquid, acidify with dilute sulphuric acid, and heat just to the point of boiling. Filter the liquid when cold and thoroughly extract the clear filtrate in a separatory funnel with the same volume of a mixture, consisting of equal parts of ether and petroleum ether. If this produces an emulsion, first withdraw the lower clear aqueous layer, add 5 grams of powdered sodium chloride, and moderately agitate the emulsified ether layer. After some time a sufficient separation of the ether layer will take place. Wash the ether solution twice with 5 cc. portions of water, pass through a dry paper, and with the aid of a current of air after addition of 1 cc. of water evaporate in a porcelain dish with moderate heat. Add to the cold aqueous residue a few drops of freshly prepared 0.05 per cent. solution of ferric chloride. A distinct blue-violet color furnishes proof of presence of salicylic acid.

Meat in which salicylic acid is detected by following the above directions is to be regarded as having been treated with salicylic acid or its compounds.

ESTIMATION OF TOXICITY OF CHEMICAL COMPOUNDS BY BLOOD HAEMOLYSIS

(Vandevelde[1])

Vandevelde originally used living cells of a variety of Allium cepa (red Brunswick onion), the cell membrane of which is rich in anthocyan. The presence of this substance obviated the necessity of using a special coloring-matter in determining plasmolytically the toxicity of alcohols, ethereal oils and other substances.[2] Vandevelde recently recommended determining the toxicity of chemical compounds by blood haemolysis, using for this purpose defibrinated ox-blood (see pages 418 and 419). To establish toxicity of different alcohols, the concentration at which haemolysis just ceases is determined. A solution, in which blood-corpuscles are not hydrolyzed after a definite time but are hydrolyzed upon addition of the slightest trace of the substance examined, is a non-toxic solution for blood-corpuscles, called by Vandevelde a "critical solution."

The estimation requires:

1. Solution of 0.9 per cent. sodium chloride in 50 per cent. alcohol by volume.[3]
2. Aqueous 0.9 per cent. sodium chloride solution.
3. Suspension of 5 per cent. defibrinated ox-blood in 0.9 per cent. aqueous sodium chloride solution.

[1] A. J. J. Vandevelde: Determination of Toxicity of Chemical Compounds by Blood Haemolysis. Chemiker-Zeitung 29 (1905), 565 and 975.

[2] Bulletin de l'assoc. Belge d. Chim. 17, 253.

[3] Alcohol of this description has at 15° a sp. gr. of 0.9348.

These experiments, as in biological determinations with serum and blood suspensates, should be carried on in rather small test-tubes of uniform diameter. First put in each of several tubes 2.5 cc. of the mixtures previously prepared of aqueous and alcoholic sodium chloride solution (1 and 2) and then 2.5 cc. of the suspended blood (3), so that the total volume of the mixture in each case is 5 cc. In the mixtures the concentration of alcohol is so chosen that in successive tubes the alcohol-content increases by 5 per cent. by volume. By experiment it was established that the end-point of appearance of haemolysis could be placed at 3 hours.

Vandevelde's experiments with alcohol gave the following results:

Suspended blood in cc.	Alcoholic NaCl solution in cc.	Aqueous NaCl solution in cc.	Alcoholic concentration of mixture in vol. p.c.	After 3 hours
2.5	2.20	0.30	22.0	Haemolysis
2.5	2.15	0.35	21.5	Haemolysis
2.5	2.10	0.40	21.0	Haemolysis
2.5	2.05	0.45	20.5	Haemolysis
2.5	2.00	0.50	20.0	Haemolysis
2.5	1.95	0.55	19.5	No haemolysis
2.5	1.90	0.60	19.0	No haemolysis

These tests were repeated many times, not only with human blood but with that of other animals, and the same results were always obtained. In the above table it is shown that the solution containing 20 per cent. of alcohol by volume produces haemolysis after 3 hours and consequently is toxic. The limiting solution containing 19.5 per cent. of alcohol by volume is non-toxic and the time-limit of the critical solution is 3 hours. Since a great many experiments have shown that this limiting solution remains unchanged between 3–12 hours, 3 hours was taken as the end-point of appearance of haemolysis.

Consequently the critical solution of alcohol is one containing 19.5 cc. of absolute alcohol ($C_2H_5.OH$) in 100 cc., or 15.489 grams of $C_2H_5.OH$ in 100 cc. Since the specific gravity of absolute alcohol is 0.7943, the weight of 19.5 cc. is $19.5 \times 0.7943 = 15.489$ grams.

According to Vandevelde's experiments, addition of methyl alcohol diminished the toxicity of alcohol, whereas higher alcohols were found to be more toxic than the latter. If the toxicity of 100 parts by weight of alcohol is taken as 100, then 47 parts of isopropyl by weight, 29 of isobutyl and 12.5 of amyl alcohol are isotoxic with that quantity of alcohol. Using the plasmolytic method with onion cells, Vandevelde obtained the following results with the same series:

100 36.8 21.2 12.6

The haemolytic method is easily carried out in test-tubes and does not require use of the microscope. The form of tube, especially its diameter, is quite important in these experiments. Speed of haemolysis increases with diameter of tube.

The quantity of blood-corpuscles is of slight influence except in narrow tubes and at the beginning of the reaction. Vandevelde applies the term "critical coefficient" to the number giving the concentration of a substance necessary to kill the cells.

Physiological Salt Solution and Haemolysis

To prevent red blood-corpuscles from changing volume in experiments requiring dilution of blood, an isotonic salt solution must be used. What is an isotonic solution? If *n* gram-molecules of a body *A* are dissolved in a definite volume of solvent and *n* gram-molecules of a body *B* are dissolved in an equal volume of the same solvent, certain properties of the original solvent are changed equally in both cases. The freezing-point of the solutions is lowered and the boiling-point raised equally. The two solutions have the same vapor tension and the same osmotic pressure. In other words they are isotonic. Blood-corpuscles retain their volume unchanged, if brought into a salt solution having the same osmotic pressure as the blood-serum. Such a salt solution is isotonic with blood-serum. In the case of human and mammalian blood an isotonic solution of sodium chloride has a concentration of 9 per thousand physiological salt solution. Such a solution formerly contained 0.6 per cent. of sodium chloride. Blood-corpuscles give up water to solutions of higher concentration than 0.9 per cent. NaCl (hyperisotonic solutions) until osmotic equilibrium is established. They shrivel and hence have smaller volume. On the other hand, blood-corpuscles in salt solutions of lower concentration (hypisotonic solutions) take up water and become distended. In diluting blood with water, this swelling may go far enough to cause haemoglobin to separate from the stroma and pass into the aqueous solution. This process is called haemolysis. Alternate freezing and thawing of blood may produce haemolysis. Various chemical substances, that act as protoplasmic poisons, cause the same result. Such substances are ether, alcohol, chloroform, alkalies, bile-acids, solanine, etc. Saponins are also powerful haemolytic agents. Finally, those globulicidal substances, or haemolysins, normally occurring in blood-sera as well as those produced in immunization, belong to this class.

USE IN TOXICOLOGICAL ANALYSIS OF AQUEOUS CHORAL HYDRATE SOLUTION AS A SOLVENT FOR ALKALOIDS, GLUCOSIDES AND BITTER PRINCIPLES

(Mauch)

(Communication from Professor E. Schaer's Institute, Strassburg)

One part of water at 17.5° dissolves 4 parts of chloral hydrate, forming a very mobile solution easily filtered and capable of being kept for a long time without decomposition. This 80 per cent. solution of chloral hydrate easily dissolves relatively large quantities of alkaloids and glucosides without altering them chemically. At 17.5° one part of each of the following substances requires for solution the number of parts of solvent stated in the table:

	80 per cent. chloral hydrate solution	Water	Ether	Chloroform
Atropine	5	600	50	3.5
Quinine	6	2000	freely soluble	2
Cocaine	5	700	freely soluble	freely soluble
Morphine	5	5000	1250	100
Santonin	4	5000	125	4
Strychnine	6.5	6600	1300	6
Brucine	6.5	....	....	....
Veratrine	7.5	....	....	....

Caffeine is the only alkaloid that forms a molecular compound with chloral hydrate soluble in water. If a chloral hydrate solution of an alkaloid, freshly prepared in the cold, is diluted with considerable water, the unchanged alkaloid is precipitated almost quantitatively, for instance, morphine, strychnine and quinine. Substances like picrotoxin, santonin and acetanilide behave similarly. But when such solutions stand for a long time at ordinary temperatures, or are heated for 1–2 hours, chloral hydrate is decomposed by the vegetable base into chloroform and formic acid. Since the alkaloidal salts of formic acid are soluble in water, dilution with this solvent does not precipitate the alkaloids. Mauch has clearly shown that atropine, brucine, quinine, cocaine, morphine, narcotine, strychnine and veratrine behave as described.

In tests ordinarily made with the ether or chloroform residue, Mauch recommends dissolving the residue in 80 or 60 per cent. chloral hydrate solution. This "chloral solution" should be of great value in color tests that depend upon use of pure sulphuric acid, or sulphuric acid containing iron or molybdic acid. These solutions contain so little water that it cannot modify the action of sulphuric acid upon the substance in solution. Such a "chloral solution" is also well suited for zone tests. An aqueous solution forms an upper layer with the "chloral solution," and the latter forms an upper layer with concentrated sulphuric acid.

Specific gravity of 80 per cent. chloral hydrate solution = 1.514
Specific gravity of 60 per cent. chloral hydrate solution = 1.3535

In tests ordinarily performed in test-tubes, it is advisable to use small tubes (6 or 7 cm. high; 1 cm. in diameter) holding 6 cc. They should not be made of too thin glass. The chloral solution cannot be used in detecting picrotoxin, because chloral hydrate produces the same reduction changes caused by picrotoxin. The same is true of the test for strychnine, where sulphuric acid and potassium dichromate, or any other oxidizing agent, are used. Coniine and nicotine also belong to the class of alkaloids that cannot be detected in chloral hydrate solution. Concentrated chloral hydrate solutions cannot be used directly in tests with general alkaloidal reagents, because precipitates do not appear until the solutions have been diluted with 6–8 volumes of very dilute hydrochloric or sulphuric acid.

In using the "chloral hydrate method" in toxicological analysis, the ether, chloroform or amyl alcohol extract should be evaporated with gentle heat upon a watch-glass of medium size (about 5 cm. diameter) and not too flat. Add to the residue, according to the quantity, about 3 cc. of 75 per cent. chloral hydrate solution. Cover the glass and let stand for some time. Occasionally tilt the glass and bring the solution thoroughly in contact with the residue. Pass the solution through a very small filter, if necessary, and wash both watch-glass and filter with a few drops of pure chloral hydrate solution. Use this chloral hydrate solution for individual tests. In testing for strychnine, evaporate part of the chloral solution to dryness upon the water-bath. Warm, until the residue does not smell of chloral, and then test for strychnine with sulphuric acid and potassium dichromate.

To recover from chloral hydrate solution most of the alkaloids and substances like picrotoxin, acetanilide and phenacetine, add excess of sodium hydroxide solution and extract thoroughly with a little chloroform. The "chloral hydrate method" is conducive to very neat work which is a great advantage. Use of metallic utensils such as knives and spatulas is entirely unnecessary.

PHOSPHORUS IN INFLAMMABLES

Official Directions for Chemical Examination of Inflammables for White or Yellow Phosphorus (German)

I. Preliminary Remarks

The following analytical directions are used in testing:

1. Red and light-red phosphorus, as well as phosphorus-compounds, that is, compounds of sulphur and phosphorus used in preparing ignition-mixtures.
2. Ignition-mixtures.
3. Matches and other inflammables.

Of the above materials, ignition-mixtures, matches and other inflammables are always examined by procedure III; and, if the result is positive, further examined by procedure IV.

Red phosphorus is tested by procedure III.

Procedure III is not used in examining compounds of sulphur and phosphorus or light-red phosphorus.

II. Preparation of the Sample for Examination

First dry the material, if necessary, in desiccator, moisten well with benzene, and then bring to as fine a state of division as possible, provided this can be done without danger of explosion. As a rule it is not necessary to dry matches in a desiccator. Carefully scrape the ignition-mixture from them with a knife. But if the ignition-mixture is so inflammable that it cannot be removed in this manner, cut the heads off as short as possible. Transfer material thus prepared to a flask, add benzene, connect with a reflux, and set upon a boiling water-bath for 30 minutes. For this treatment take 3 grams of phosphorus, or phosphorus-compounds, and 150 cc. of benzene; 3 grams of ignition-mixtures and 15 cc. of benzene; and 3

grams of ignition-mixture scraped from matches, or 200 match-heads, and 15 cc. of benzene. Cool the benzene solution, which may contain white or yellow phosphorus, pass through a folded filter, and use for tests below.

III. Ammoniacal Silver Nitrate Test

Add 1 cc. of benzene solution to 1 cc. of ammoniacal silver nitrate solution, prepared by dissolving 1.7 grams of silver nitrate in 100 cc. of ammonia solution (sp. gr. 0.992 = 2 per cent.).

Shake the two solutions vigorously and allow to settle. If no change takes place, or the aqueous layer acquires only a pure yellow color, white or yellow phosphorus is absent. A decision should be made at once as soon as the liquids have settled and not after long standing.

On the other hand, if after the liquids have been shaken the aqueous layer shows an immediate reddish or brown coloration, or a black or brown-black precipitate, this may be due to white or yellow phosphorus, light-red phosphorus, or phosphorus sulphide compounds. If red phosphorus is under examination, and the result of the reaction is as described, presence of white or yellow phosphorus is shown and further testing is unnecessary.

In all other cases the remainder of the benzene solution is subjected to the following procedure.

IV. Luminescence Test for White or Yellow Phosphorus

Dip a strip of filter-paper 10 cm. long and 3 cm. wide in the benzene solution until saturated. Drain off and preserve excess of solution. Attach the strip to a wire hook in a cork set in the upper end of a glass tube 50 cm. long and 4.5 cm. in diameter. Clamp this tube in a vertical position and allow the lower open end to extend about 3 cm. into the inner compartment (about 10 cm. in diameter) of a Victor Meyer heating apparatus. Adjust a thermometer in the cork at the upper end of the glass tube so that the mercury-bulb extends to within 20 cm. of the lower end of the latter. Boil water in the heating apparatus with a Bunsen burner, provided with a sheet-iron mantle to prevent as much as possible emission of light. Surround heating apparatus and burner with a sheet-iron cylinder upon which rests a cone-shaped cover also of sheet-iron. In this manner all light-rays from the flame are screened off (Fig. 57).

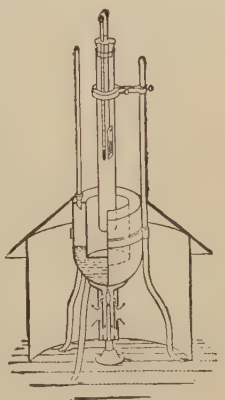


FIG. 57.—Apparatus for luminescence test.

Care should be taken in putting the cork into the glass tube not to let the paper strip dipped in the benzene solution touch the walls of the tube, or to wet the latter with the benzene solution. To permit necessary circulation of air in the tube, four V-slits should be cut in the cork holding the paper strip and thermometer. During the experiment the temperature of the air-current should be 40–50°. This may be regulated by varying the depth to which the tube projects into the inner chamber of the heating apparatus. Under no conditions should the temperature exceed 55°.

Conduct the test in a room that can be completely darkened. Care should be taken that no light from outside nor from the flame of the burner reaches the eye of the observer. Before beginning the test wait until the eye has accustomed itself to the darkness, otherwise luminescence cannot be detected with as much certainty. Such manipulations, as are necessary before the test, can best be carried out with the aid of a dim light screened off at the side of the observer. Great weight is attached to the observance of these instructions.

The test itself should not be made until the serviceability and reliability of the apparatus has first been determined by means of a benzene solution containing 1 mg. of white phosphorus in 10 cc. Care should also be taken in this preliminary test that the temperature of the air-current in the tube does not exceed the limit mentioned. Carefully clean the apparatus after it has been found to be in good working condition and make the test itself.

If the paper strip shows luminescence after 2–3 minutes, presence of white or yellow phosphorus is proved. Luminescence usually begins faintly at the upper and lower ends of the paper strip and spreads toward the middle. If a rather large quantity of phosphorus is present (about 1 mg. in 10 cc. of benzene or more), luminescence increases in intensity and shortly characteristic luminous clouds begin to rise from the paper and issue from the tube. Occasionally luminous, serpentine lines extend from the margins to the middle of the paper and then shortly luminescence covers the entire surface. In this case too appearance of luminous clouds may be observed a little later but otherwise in the same manner.

When luminescence does not appear after 2–3 minutes, the experiment should be continued for 2–3 minutes longer. A decision as to absence of white or yellow phosphorus should not be made from absence of luminescence until expiration of this time. In every case at the conclusion of the test it should be noted whether or not the temperature exceeded 55°. If so, then the test should be repeated when luminescence appears. This is also necessary, when luminescence is doubtful, indistinct or too late in appearing.

V. Test for Phosphorus Sulphide Compounds

If ammoniacal silver nitrate solution gives a positive result and it is desired, regardless of the outcome of the luminescence test, to establish whether or not phosphorus sulphide compounds are present, the following test should be made:

Add 1 cc. of twice normal aqueous lead nitrate solution to 1 cc. of the original benzene solution and thoroughly shake the mixture. When the two liquids have separated, presence of phosphorus sulphide compounds is proved, if a brown coloration appears at the contact-surface of the two liquids, or a black or brown-black precipitate of lead sulphide.

VI. Conclusion

If ammoniacal silver nitrate solution gives a positive result, but the luminescence test and that with lead nitrate solution are negative, presence of light-red phosphorus is to be accepted as proved.

LEGAL PROVISIONS AND METHODS OF DETECTING ARSENIC AND TIN IN COLORED FOODSTUFFS AND ARSENIC IN SPUN OR WOVEN GOODS

Law Pertaining to Use of Deleterious Colors in Foodstuffs and Commodities[1]

(German)

§1. Colors injurious to health shall not be used in the preparation of foodstuffs to be offered for sale.

In the sense of this provision, colors injurious to health are such dyestuffs and dye-preparations as contain antimony, arsenic, barium, lead, cadmium, chromium, copper, mercury, uranium, zinc, tin, gamboge, coralline and picric acid.

The imperial chancellor is authorized to issue more specific directions relative to the procedure to be followed in proving presence of arsenic and tin.

§2. No containers, wrappers or protective coverings, into the manufacture of which colors of the kind specified in Section 1, Paragraph 2, have entered, shall be used for storing or packing foodstuffs to be offered for sale.

This provision is not applicable to use of barium sulphate (heavy spar, blanc fixe); barium color-lakes free from barium carbonate; chromic oxide; copper, tin, zinc and their alloys as metallic colors; cinnabar; stannic oxide; tin sulphide as mosaic gold; all colors fused into glass, glazes or enamels; and colors of waterproof materials painted on the outside of containers.

§3. Substances designated in Section 1, Paragraph 2, shall not be used in the preparation of cosmetics (materials for cleansing, caring for or coloring the skin, hair or oral cavity) to be offered for sale.

This provision does not apply to barium sulphate (heavy spar, blanc fixe), cadmium sulphide, chromic oxide, cinnabar, zinc oxide, stannic oxide, zinc sulphide, and also copper, tin, zinc and their alloys in powdered form.

§4. Colors designated in Section 1, Paragraph 2, shall not be used in the manufacture of toys (including prints, picture-books and water-colors for children), trellises for flower-pots, and artificial Christmas trees to be offered for sale.

This provision does not apply to substances designated in Section 2, Paragraph 2, or to antimony sulphide and cadmium sulphide as pigments in rubber masses, lead oxide in varnish, white lead as a component of so-called wax-castings, provided however that it does not exceed 1 part by weight in 100 parts by weight of the mass, lead chromate (by itself, or in combination with lead sulphate) as an oil- or lacquer-color, or with a coating of lacquer or varnish, compounds of zinc insoluble in water, but in the case of rubber toys only when used as pigments of the rubber mass, as oil- or lacquer-colors, or with a coating of lacquer or varnish, and all colors fused into glazes or enamels.

The only requirements applicable, as far as concerns use of articles listed in Sections 7 and 8 in the manufacture of toys, are those given in Sections 7 and 8.

§5. In the manufacture of printed and lithographed articles, only such colors as do not contain arsenic shall be used upon materials designated in Sections 2, 3 and 4.

[1] Reichs-Gesetzbl., July 5th, 1877, page 277.

§6. Unless water-colors of every description meet the requirements of Section 4, paragraphs 1 and 2, they shall not be sold, or offered for sale, as free from deleterious materials or as non-toxic.

§7. Colors containing arsenic shall not be used in the manufacture of wall-papers, upholstery fabrics, carpets, materials for draperies or dress-goods, masks, candles, and artificial leaves, flowers and fruits.

This provision does not apply to use of arsenical mordants or fixatives in dyeing or printing spun or woven goods. But spun or woven goods made by using such materials shall not enter into the manufacture of articles designated in Paragraph 1, if they contain arsenic in water-soluble form, or in such quantity that more than 2 mg. of arsenic is present in 100 sq. cm. of finished goods. The imperial chancellor is authorized to issue specific directions as to the procedure to be used in determining the quantity of arsenic.

§8. The requirements of Section 7 also apply to manufacture of writing-materials, lamp-shades and screens, as well as candle-shades.

Provisions in Section 1 apply to manufacture of sealing-wafers, but only when used in connection with foods, although barium sulphate (heavy spar, blanc fixe), chromic oxide and cinnabar are permitted.

§9. Water-colors or size-colors containing arsenic shall not enter into the manufacture of paint for floors, ceilings, walls, doors and windows of rooms used for living or business purposes, roll-shutters, ventilator-shutters, folding-shutters or blinds, furniture and other household articles.

§10. Provisions of Sections 2–9 do not apply to use of colors containing the substances designated in Section 1, Paragraph 2, when present not as regular components but only as impurities, such as cannot be avoided in the ordinary course of manufacture.

§11. The provisions of this law are not applicable to the dyeing of furs.

§12. Infringement of this law in the following cases shall be punishable by fine of 150 gold Marks or imprisonment:

1. Whoever, in violation of the provisions of Sections 1–5, 7, 8 and 10, manufactures, preserves or packs foods, condiments or commodities, or sells commercially such manufactured, preserved or packed commodities, or offers them for sale.

2. Whoever violates the provisions of Section 6.

3. Whoever violates the provisions of Section 9 and also whoever sells commercially, or offers for sale, articles manufactured in violation of Section 9.

§13. In addition to the fine provided for in Section 12, the commodities illegally manufactured, preserved, packed, sold or offered for sale shall be confiscated, regardless of whether they belong to the guilty party or not.

When sentence cannot be imposed upon any particular person, it is to be understood that confiscation shall be carried out.

§14. The provisions of the law of May 14th, 1879 (Reichs-Gesetzblatt, page 145), relative to traffic in foods, condiments and commodities, remain in effect.

The provisions in Sections 16 and 17 of this law apply also to violations of the provisions of the present law.

§15. This law goes into effect May 1st, 1888, and on the same day imperial regulations, of May 1st, 1882, relative to use of poisonous colors, become obsolete.

A. TESTS FOR ARSENIC AND TIN IN COLORED FOODS OR CONDIMENTS

I. Solids

1. Take for the test 20 grams of a solid food-material colored throughout, or if superficially colored scrape off an amount of color corresponding to 20 grams of material. Less material should be used only when the quantity specified is not available.

2. Reduce the sample of a fine state of division by grinding, or otherwise, and treat in a porcelain dish with a measured quantity of a pure hydrochloric acid (sp. gr. 1.10–1.12 = 20–24 per cent.) and enough distilled water to make the ratio of acid to water about 1:3 Usually 25 cc. of acid and 75 cc. of water answer the purpose.

Now add 0.5 gram of potassium chlorate, set the dish upon a water-bath, and add (as soon as the contents have come to the temperature of the bath) small portions of potassium chlorate at 5–15 minute intervals, until the liquid is thin, light yellow and uniform. Usually 2 grams of this salt answer the purpose. Replace water from time to time as it evaporates. When the specified point has been reached, add 0.5 gram more of potassium chlorate and remove the dish from the bath. Cool thoroughly, transfer the contents of the dish to a filter, and allow all of the liquid to run into a 400 cc. flask. Heat upon the water-bath until the smell of chlorine is gone. Thoroughly wash filter and residue, which usually appears, with hot water and evaporate the wash-water upon the water-bath to about 50 cc. Combine this liquid and any precipitate with the main filtrate. The total volume of liquid should be at least six times that of the hydrochloric acid used. If, for example, 25 cc. of hydrochloric acid were used, the combined filtrate and wash-water should amount at least to 150 cc., or better 200–250 cc.

3. Keep the liquid at 60–80° and for 3 hours pass in a slow stream of pure, washed hydrogen sulphide. Then allow the liquid to cool, all the while passing in the gas. Cover the flask lightly with filter-paper and allow to stand in a moderately warm place for at least 12 hours.

4. If there is a precipitate, transfer to a filter, and wash with water containing hydrogen sulphide. Then treat while still moist with moderately yellow ammonium sulphide previously diluted with ammonia. Usually 4 cc. of ammonium sulphide, 2 cc. of ammonia (about 0.96 sp. gr. = 10 per cent.), and 15 cc. of water will answer the purpose. Wash the residue from the ammonium sulphide treatment with water containing ammonium sulphide, and evaporate filtrate and wash-water at gentle heat to dryness in a deep, 6 cm. porcelain dish. Pour about 3 cc. of red, fuming nitric acid over the residue, cover the dish with a clock-glass, and evaporate cautiously at gentle heat. If the residue while moist is yellow, it may be treated as described below. But if dark, treat again with red, fuming nitric acid, until yellow in the moist state.

5. Add finely ground sodium carbonate to the moist residue until the reaction is strongly alkaline, add 2 grams of a mixture of 3 parts of sodium carbonate and 1 part of sodium nitrate, and mix with a little water to form a uniform paste-like mass. Dry this mass in the dish and heat carefully to the point of sintering, or incipient fusion. Do not carry the temperature higher. The resultant mass is

white and colorless. If this is not the case, add more sodium nitrate until the mass is in this condition.

6. Soften the mass with water at gentle heat and filter through moist paper. If tin is present, it will be in the residue upon the filter as white stannic oxide, whereas arsenic will be in the filtrate as sodium arsenate. If there is a residue upon the filter, it should be borne in mind that small quantities of tin may pass into the filtrate. Wash the residue once with cold water and three times with a mixture of equal parts of water and alcohol. Evaporate the washings so that, when combined with the filtrate, the total volume is about 10 cc. Add dilute nitric acid drop by drop until the liquid just reacts acid. Should a slight precipitate of hydrated tin oxide appear, collect on a filter and wash as described above. Further directions for detection of tin are given under 10.

7. The first step in the detection of arsenic consists in forming ammonium arseno-molybdate. The clear solution, acidified as directed above with nitric acid, should have a volume of about 15 cc. It is freed from carbon dioxide and nitrous acid, by warming, cooled and filtered if necessary. Then mix in a flask with about an equal volume of a solution of ammonium molybdate in nitric acid and allow to stand for 3 hours without warming. If, as a result of incomplete washing of the hydrogen sulphide precipitate, the solution contains some phosphoric acid, this will separate as ammonium phospho-molybdate. This will not occur, however, when directions are carried out more carefully.

8. Warm the clear or filtered liquid upon the water-bath, until after about 5 minutes it has come to the temperature of the bath. If arsenic is present, a yellow precipitate of ammonium arseno-molybdate will appear and usually some white molybdic acid will separate at the same time. After the liquid has stood for 1 hour, decant upon a small filter from the main part of the precipitate in the flask. Wash the precipitate twice with small quantities of a mixture of 100 parts of ammonium molybdate solution, 20 parts of nitric acid (sp. gr. 1.2 = 32 per cent.), and 80 parts of water. Dissolve with heat in 2–4 cc. of aqueous ammonia (sp. gr. 0.96 = 10 per cent.) and add about 4 cc. of water. Pour, if necessary, again through the small filter, add one-fourth the volume of alcohol, and 2 drops of a solution of ammonium magnesium chloride. Arsenic immediately separates, or after standing in the cold, as white ammonium magnesium arsenate which is more or less crystalline. Collect on a filter and wash with as small a quantity as possible of a mixture of 1 part of ammonia, 2 parts of water and 1 part of alcohol.

9. Dissolve the precipitate in as small a quantity of dilute nitric acid as possible and evaporate this solution to a very small volume. Place one drop upon a porcelain dish and another upon an object-glass. Add to the first a drop of a solution of silver nitrate and then at the margin a drop of aqueous ammonia (sp. gr. 0.96 = 10 per cent.). If arsenic is present, a red-brown streak of silver arsenate will form at the juncture of the two liquids. Render the drop upon the slide alkaline with as small a quantity of aqueous ammonia as possible. If arsenic is present, a precipitate of ammonium magnesium arsenate will appear at once or very soon. Under the microscope this precipitate will appear to be made up of small, lance-like crystals.

10. To test for tin, dry the filter-paper or papers containing stannic oxide, incinerate in a porcelain crucible and weigh. Only when the residue, after subtrac-

tion of the weight of the filter ash, amounts to more than 2 mg. should a further examination for tin be made. In this case put the residue into a porcelain boat, introduce the latter into a tube of difficultly fusible glass, drawn out at the forward end to a long tip with a fine opening, and heat in a stream of pure, dry hydrogen, gradually increasing the temperature until no more water appears and all stannic oxide is reduced. Allow the boat to cool in the stream of hydrogen and then remove from the tube. Incline the boat slightly, add a few drops of hydrochloric acid (sp. gr. 1.10–1.12 = 20–24 per cent.) to the lower part, and return to the tube. Continue passing in a slow stream of hydrogen, incline the tube so that the hydrochloric acid in the boat comes in contact with the reduced tin, and warm slightly. Tin is dissolved in the hydrochloric acid, forming stannous chloride with evolution of hydrogen. Cool in a stream of hydrogen and remove the boat from the tube. If necessary, add a few drops more of a mixture of 3 parts of water and 1 part of hydrochloric acid. Test a drop of the solution obtained for tin with mercuric chloride, gold chloride and hydrogen sulphide, using the latter both before and after addition of a little bromine-hydrochloric acid or chlorine water.

If, after treatment of the contents of the boat in the manner described above, a black residue insoluble in hydrochloric acid remains, it may be due to antimony.

II. Liquids, Fruit Jellies and Similar Products

11. In the case of liquids, fruit jellies, etc., take a weight of material that will amount to about 20 grams when dry. This will require about 30 grams of raspberry syrup, 35 grams of currant jelly, and about 800–1000 grams of red wine, vinegar, etc. A smaller quantity should be taken for the test only when the amount stated is not available.

12. Treat fruit juices, jellies, etc., with hydrochloric acid, potassium chlorate, etc., exactly as described in Section I. Thin liquids that do not react acid should be concentrated to a small volume by evaporation and treated with hydrochloric acid, potassium chlorate, etc., as directed in Section I. Thin liquids that do react acid should be distilled to a smaller volume and then treated with hydrochloric acid, potassium chlorate, etc., as directed in Section I. Also add hydrochloric acid to the distillate and then treat with hydrogen sulphide. Combine any precipitate that may appear with that obtained according to No. 3.

B. TESTS FOR ARSENIC IN SPUN OR WOVEN GOODS

13. Cut 30 grams of the given material into small pieces and extract for 3–4 hours with distilled water at 70–80°. Filter the liquid and thoroughly wash the residue. Evaporate filtrate and wash-water to about 25 cc., cool, add 5 cc. of pure concentrated sulphuric acid, and test the liquid in the Marsh apparatus for arsenic, using arsenic-free zinc.

If an arsenic mirror is obtained, then the spun or woven goods contain arsenic in water-soluble form.

14. If the test in No. 13 is negative, take 10 grams of material and determine its area. The area of spun goods is determined by comparison with another textile made of the same kind of yarn having the same strength of fibre.

15. If the available material is not sufficient for Nos. 13 and 14, smaller quantities should be taken, that is, in the case of No. 14 the material extracted with water for the test in No. 13 may be dried and used.

16. Cut the yarn or goods into small pieces, put into a 400 cc. tubulated retort of potassium glass, and add 100 cc. of pure hydrochloric acid (sp. gr. 1.19 = 37.9 per cent.). Draw out the neck of the retort and bend at an obtuse angle. Then set the retort so that the part of the neck next to the bulb is directed obliquely upward and the other end obliquely downward. Attach the latter to a Liebig condenser and make a tight connection with a piece of rubber tubing. Make an air-tight connection between the condenser-tube and a 500 cc. tubulated receiver. Put about 200 cc. of water into the latter and immerse in a dish of cold water. Connect the tubulus of the receiver with a Péligot-tube containing water.

17. At the end of 1 hour introduce into the retort 5 cc. of a solution prepared from crystallized, arsenic-free ferrous chloride and apply heat. As soon as excess of hydrochloric acid has passed over, raise the temperature until the liquid boils, and distil until the contents begin to swell considerably. Cool, add 50 cc. more hydrochloric acid of sp. gr. 1.19 to the retort and distil again in the same manner.

18. Combine the contents of the receiver, the color of which is brown on account of organic matter, with the solution in the Péligot-tube, dilute with distilled water to about 600–700 cc., and pass in hydrogen sulphide both when the solution is warm and after it is cold.

19. After 12 hours filter the brown precipitate, consisting partially or wholly of organic substances, upon a filter prepared by depositing asbestos in a funnel, the tube of which is provided with a glass stop-cock. Wash the precipitate with a little water, close the cock, treat the precipitate in the funnel with a few cc. of bromine-hydrochloric acid, prepared by dissolving bromine in hydrochloric acid (sp. gr. 1.19 = 37.9 per cent.), and cover with a glass plate or watch-glass. After the reagent has acted about 30 minutes, open the cock, and allow the solution to run into the precipitation flask, upon the walls of which frequently a small portion of the hydrogen sulphide precipitate adheres. Wash the residue upon the asbestos filter with hydrochloric acid of sp. gr. 1.19.

20. Again add to the solution in the flask excess of ferrous chloride, and transfer to a smaller retort of a second distilling apparatus, rinsing out the flask with hydrochloric acid of sp. gr. 1.19. In other respects the procedure is the same as described in No. 16. Distil as directed in No. 17, carrying distillation as far as possible. Cool, add another 50 cc. portion of hydrochloric acid of sp. gr. 1.19 to the retort, and again distil.

21. The distillate usually is water-clear. Dilute to about 700 cc. with distilled water and pass in hydrogen sulphide as described in No. 18. After 12 hours collect any precipitate of arsenious sulphide upon a small paper, that has been successively washed with dilute hydrochloric acid, water and alcohol, dry at 110° and weigh. Wash the residue upon the filter first with water, then with absolute alcohol, warm carbon disulphide and finally with absolute alcohol. Dry at 110° and weigh.

22. Calculate from the weight of arsenious sulphide obtained the quantity of arsenic and then ascertain the amount of arsenic in 100 sq. cm. of the spun or woven goods upon the basis of the determination of area made in No. 14.

CHAPTER VI

QUANTITATIVE ESTIMATION OF ALKALOIDS AND OTHER PRINCIPLES

Estimation of Alkaloids in Drugs and Pharmaceutical Preparations

(German Pharmacopoeia)

Alkaloids are nitrogenous bases occurring in plants. The term "plant base" is synonymous with alkaloid. The plant families especially rich in alkaloids are berberideae, cinchonaceae, papaveraceae, solanaceae, and strychnaceae. Alkaloids as a rule are not uniformly distributed in all parts of plants. They occur most often in roots, fruits and seeds. If the plant is a tree, there is often more alkaloid in the bark than in other parts. The particular part of the plant usually contains only a few per cent. of alkaloid. Quinine bark is an exception, the quantity of alkaloid being 5–10 per cent. and sometimes more. Plants as a rule do not contain free alkaloids but their salts. They are combined not only with the mineral acids, sulphuric, hydrochloric, and possibly phosphoric, but with organic acids, such as malic, aconitic, tannic, citric, quinic, and meconic. Most free plant bases dissolve only slightly in water but readily in alcohol, ether and chloroform. The German Pharmacopoeia prescribes ether, chloroform, or a mixture of these solvents, for extraction of free alkaloids. The finely powdered drug should first be treated with a solution of sodium hydroxide, ammonia, or sodium carbonate, to liberate alkaloids from their salts:

$$\begin{matrix} C_{20}H_{24}N_2O_2 \\ | \\ C_6H_7(OH)_4COOH \end{matrix} + NaOH = C_{20}H_{24}N_2O_2 + C_6H_7(OH)_4COONa + H_2O$$

Quinine quinate — Quinine — Sodium quinate

Sodium bicarbonate, calcium and barium hydroxide, also decompose salts of most alkaloids, giving the free bases.

The chloroform-ether mixture removes not only free alkaloids from the drug but varying amounts or other substances, such as fat, resin, wax, phytosterol, and pigments. To free the alkaloid from such impurities, shake the chloroform-ether extract with a measured excess of 0.1 or 0.01 n-hydrochloric acid. The alkaloid passes into aqueous solution as hydrochloride:

$$C_{20}H_{24}N_2O_2 + HCl = C_{20}H_{24}N_2O_2.HCl$$

Quinine — Quinine hydrochloride

But most of the impurities remain in the chloroform-ether mixture. Finally, determine excess of hydrochloric acid by titration of the aqueous solution with 0.1 or 0.01 n-sodium hydroxide solution, employing usually iodeosine or haematoxylin as indicator. Calculate amount of alkaloid in the drug from the difference between the original quantity of acid and the excess.

Estimation of alkaloids in drugs, other raw materials, and pharmaceutical preparations, requires according to directions of the German Pharmacopoeia the following steps:

1. Liberation of alkaloids from salts by means of stronger bases, such as potassium and sodium hydroxide, ammonia and sodium carbonate.

2. Extraction of free alkaloids with ether, or better with chloroform-ether mixture.

3. Transference of alkaloids from ether, or chloroform-ether, to aqueous 0.1 or 0.01 n-hydrochloric acid solution always present in excess.

4. Determination of excess of hydrochloric acid in an aliquot volume, usually 50 cc. of the hydrochloric acid solution of the alkaloid diluted to 100 cc., by titration with 0.1 or 0.01 n-sodium hydroxide solution.

Only in certain cases is the alkaloid determined gravimetrically rather than volumetrically, for example, in estimating hydrastine in hydrastis root and in its fluid extract. Morphine, almost insoluble in its crystallized form in ether and only very slightly soluble in a chloroform-ether mixture, is precipitated in this condition by ammonia and, after filtration, washing and drying, is dissolved in excess of 0.1 n-hydrochloric acid.

Volumetric Estimation of Alkaloids

(German Pharmacopoeia)

Most alkaloids are such strong bases that they can be determined volumetrically by the alkalimetric method. They are determined differentially by dissolving the alkaloidal residue in excess of 0.1 or 0.01 n-hydrochloric or sulphuric acid and titrating excess of acid with 0.1 or 0.01 n-sodium hydroxide solution, using iodeosine as indicator.

Procedure.—In a legal-chemical investigation the author first weighs an alkaloidal residue even though impure. The ether or chloroform solution to be used for this gravimetric determination, after several hours standing in a small dry flask, is passed through a dry filter into a small weighed dry flask. Evaporation is conducted at gentle heat and the residue dried to constant weight in desiccator over sulphuric acid. A larger, bell-shaped desiccator shown elsewhere (Fig. 13, page 96) has been found convenient for this purpose. In the examination of cadaveric material, gravimetric determinations of alkaloids always give too high results, for even with the most careful manipulation it is almost impossible to get absolutely pure ether or chloroform extracts of alkaloids. Yet the approximate figures obtained are in many cases of value to the forensic chemist. For the volumetric determination, the weighed alkaloidal residue should be dissolved in a measured excess of 0.1 or 0.01 n-hydrochloric or sulphuric acid. The solution, if necessary, should be passed through a small filter into a 250 cc. glass-stoppered flask of pure white glass[1] poor in alkali. The solution is then diluted with 50 cc. of water and acid-free ether added to form a layer about 1 cm. in depth. After addition of 10 drops of iodeosine solution, the mixture is shaken. During agitation 0.1 n-sodium hydroxide solution is run in drop by drop, or 0.01 n by the cc.,

[1] The author uses for this purpose flasks that have stood for a day in contact with 0.1 n-hydrochloric acid. After the acid has been poured out, steam is then passed through.

until after vigorous shaking the aqueous layer just has a distinct rose color. Should too much alkali be added, 0.1 n-acid is again added in slight excess and 0.1 n-sodium hydroxide solution carefully run in drop by drop until after shaking the aqueous layer has a persistent faint rose-red tint.

Most of the better known alkaloids behave like monacid bases toward the indicator iodeosine, for example, atropine, hyoscyamine, scopolamine, brucine, cocaine, coniine, morphine, nicotine, pilocarpine, and strychnine. The equivalent weight of each of these alkaloids is the same as its molecular weight. So 1000 cc. of 0.1 n-hydrochloric or sulphuric acid show a 0.1-gram-equivalent weight of alkaloid, and 1 cc. of one of these acids neutralizes a 0.1-mg. equivalent weight of alkaloid.

1000 cc. of 0.1 n-acid indicate of

Atropine (Hyoscyamine)	0.1 $C_{17}H_{23}NO_3$	grams =	28.9 grams
Brucine	0.1 $C_{23}H_{26}N_2O_4$	grams =	39.4 grams
Cocaine	0.1 $C_{17}H_{21}NO_4$	grams =	30.3 grams
Coniine	0.1 $C_8H_{17}N$	grams =	12.7 grams
Morphine	0.1 $C_{17}H_{19}NO_3$	grams =	28.5 grams
Nicotine	0.1 $C_{10}H_{14}N_2$	grams =	16.2 grams
Pilocarpine	0.1 $C_{11}H_{16}N_2O_2$	grams =	20.8 grams
Strychnine	0.1 $C_{21}H_{22}N_2O_2$	grams =	33.4 grams

This method of determination is not applicable to very weak bases the salts of which undergo marked hydrolysis in aqueous solution. Such alkaloids are hydrastine, caffeine, narceine, narcotine, papaverine, theobromine, theophylline, and the ergot alkaloids.

The strong bases of cinchona bark, especially quinine and cinchonine as well as their isomers can be titrated with 0.1 n-acid only with haematoxylin as indicator. The cinchona alkaloids behave as monacid bases only in presence of this indicator.

Estimation of Alkaloids by Picrolonate Method

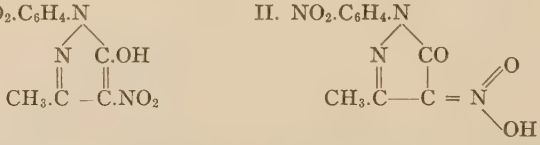
(Matthes and Rammstedt[1])

Knorr[2] gave the name picrolonic acid to 1-p-nitrophenyl-3-methyl-4-isonitropyrazolone. This compound is formed by the action of nitric acid upon methylphenyl-pyrazolone. In properties picrolonic acid resembles picric acid and is characterized by forming crystalline salts with many organic bases, such as the alkaloids. These salts as a rule dissolve with difficulty, are well crystallized, have a yellow to red color, and undergo decomposition when heated. Picrolonic acid is frequently of service in characterizing bases. Hydrochloric acid precipitates this compound from a solution of its sodium salt as a yellow, mealy powder, melting when rapidly heated at about 128°, becoming dark in color and

[1] H. Matthes and O. Rammstedt: Use of Picrolonic Acid in Estimating Strength of Narcotic Drugs, Extracts and Tinctures. Arch. d. Pharm. 245 (1917), 112; Use of Picrolonic Acid (Dinitro-phenyl-methyl-pyrazolone) for Quantitative Estimation of Certain Alkaloids. Zeitschr. f. analyt. Chem. 46 (1907), 565.

[2] L. Knorr: Ber. d. Deutsch. Chem. Ges. 30, 909; and 32, 732.

undergoing complete decomposition with rapid evolution of gas. Knorr first gave picrolonic acid formula I but at present formula II[1] is preferred:



Matthes estimated many alkaloids quantitatively by means of picrolonic acid. Collect the precipitated alkaloidal picrolonate upon a weighed Gooch crucible, wash, dry and weigh. Estimation of alkaloids by this method is possible because picrolonates are constant in composition. Morphine, hydrastine, codeine, strychnine, brucine, pilocarpine and stypticine[2] may be estimated quantitatively by this method.

Estimation of Morphine, Codeine and Stypticine in Solutions, Tablets and Sugar Triturates

Dissolve the weighed sugar triturate, or tablet, in the smallest quantity of water possible and add picrolonic acid solution (about 0.1 n-solution in alcohol) in slight excess. The picrolonate separates at once, or very soon, as yellow crystals or a crystalline meal. Allow to stand 15–30 minutes at room temperature, or better in an ice-box, and collect the precipitate upon a weighed Gooch crucible. Wash with a little ice water, dry 30 minutes at 110° and weigh. Morphine picrolonate usually separates in 10–30 minutes. Cooling in an ice-box aids precipitation.

	Formula	Mol. Wt.	Decomposition-point
Morphine picrolonate. . .	$C_{17}H_{19}NO_3.C_{10}H_8N_4O_5$	549	200–210°
Codeine picrolonate.	$C_{18}H_{21}NO_3.C_{10}H_8N_4O_5$	563	about 225°
Cotarnine picrolonate. . .	$C_{12}H_{15}NO_4.C_{10}H_8N_4O_5$	501	205–210°

Notes.—In these determinations do not use too dilute solutions of alkaloids and do not wash the picrolonate precipitates with too much water. In comparative examinations picrolonate precipitations should always be carried out under the same conditions. Dissolve powdered morphine and sugar mixture in about 5–10 cc. of water. Matthes and Rammstedt in examining the morphine powder obtained the following results:

Weights taken: 0.019 gram morphine hydrochloride + 0.5 gram sugar.

Results obtained:

I. 0.0273 gram morphine picrolonate = 0.0187 gram morphine hydrochloride.

II. 0.0274 gram morphine picrolonate = 0.0187 gram morphine hydrochloride.

In a second experiment each 10 cc. portion of an aqueous solution contained 0.0104 gram of morphine hydrochloride.

Results obtained:

I. 0.0147 gram morphine picrolonate = 0.0101 gram morphine hydrochloride.

[1] R. Zeine: Inaug.-Diss., Jena, 1906.

[2] Stypticine is cotarnine hydrochloride, $C_{15}H_{10}NO_4.HCl.H_2O$.

II. 0.0146 gram morphine picrolonate = 0.0099 gram morphine hydrochloride.[1]

The picrolonic acid method may also be used in estimating various narcotic drugs, extracts and tinctures; with especial advantage in determining alkaloids in nux vomica (page 621), hydrastis root (page 606), jaborandum leaves (page 615), and their pharmaceutical preparations. Alkaloid extracted in the usual manner (see Directions of German Pharmacopoeia) is treated in ether-chloroform solution with 0.1 n-alcoholic picrolonic acid solution. After 24 hours the picrolonate precipitate is collected upon a Gooch crucible, washed with 2 cc. of an alcohol-ether mixture (1 + 3), dried for half an hour at 110° and weighed.

	Mol. Wt.	Decomposition-point
Brucine picrolonate	658	277°
Strychnine picrolonate	598	286°
Hydrastine picrolonate	647	220°
Pilocarpine picrolonate	472	200–205°

In case of nux vomica preparations, the picrolonate method gives more reliable results than the method of the German Pharmacopoeia.

Estimation of Alkaloids with Potassium Bismuthous Iodide and Decomposition of Precipitate with Alkaline Hydroxide and Carbonate

(Thoms[2])

Dissolve the particular alkaloid in sulphuric acid and precipitate completely with potassium bismuthous iodide prepared as described by Kraut.[3] Decompose the precipitate with a mixture of sodium carbonate and hydroxide, extract free alkaloid with ether and weigh. By this method Thoms recovered atropine, hyoscyamine, scopolamine, strychnine, quinine, caffeine and antipyrine from potassium bismuthous iodide precipitates unaltered and nearly quantitatively. He also used this method with success in estimating quantitatively alkaloids in belladonna extract.

Procedure.—Dissolve the alkaloidal salt, or 2 grams of belladonna extract, in 50 cc. of water. Add first 10 cc. of 10 per cent. sulphuric acid, stir and precipitate with 5 cc. of potassium bismuthous iodide solution. Collect the precipitate upon a dry filter and wash twice with 5 cc. portions of 10 per cent. sulphuric acid. Transfer the thoroughly drained precipitate and paper to a wide-mouth extraction cylinder having a tight glass stopper. Add 0.3 gram of sodium sulphite, then 30 cc. of 15 per cent. sodium hydroxide solution and shake. Add quickly 15 grams of sodium chloride and 100 cc. of ether. Shake frequently and

[1] In a private communication Professor Matthes states that with smaller quantities of morphine than 0.005 gram the picrolonate method gives less satisfactory results.

[2] H. Thoms: Estimation of Narcotic Extracts from a Chemical and Pharmacological Point of View. Ber. d. Deutsch. pharm. Ges. 13 (1903), 240; Use of Potassium Bismuthous Iodide Solution in Estimating Alkaloids. 15 (1905), 85; D. Jonescu: Precipitation and Quantitative Estimation of Alkaloids with Potassium Bismuthous Iodide Solution. Ber. d. Deutsch. pharm. Ges. 16 (1906), 130.

[3] See "Preparation of Reagents," page 639.

allow to stand for 3 hours. Ether contains the alkaloid and settles well. Remove with a pipette 50 cc. of ether solution (= half the solution of alkaloidal salt, or 1 gram of belladonna extract) and titrate this ether solution in a flask in the usual manner with 0.01 n-hydrochloric acid, using iodeosine as indicator.

After titrating belladonna alkaloids, use in the calculation the equivalent weight of atropine-hyoscyamine, $C_{17}H_{23}NO_3 = 289$. 1000 cc. of 0.01 n-hydrochloric acid correspond to 2.89 grams of atropine-hyoscyamine. Atropine and hyoscyamine being isomeric monacid bases, their formula weight and equivalent weight are the same.

Quinine, caffeine and antipyrine were also recovered unaltered from potassium bismuthous iodide precipitates. After decomposition of the precipitates, they were obtained almost quantitatively but were estimated gravimetrically.

Dissolve 2 grams of quinine[1] in 50 cc. of water acidified with sulphuric acid and precipitate with potassium bismuthous iodide. Filter the precipitate with suction and wash with 5 per cent. sulphuric acid. Transfer precipitate and paper to an extraction cylinder (see above) and shake thoroughly with a mixture of 20 grams of crystallized sodium carbonate and 40 cc. of 10 per cent. sodium hydroxide solution. The intensely yellowish red precipitate gradually becomes white. Add 30 cc. of ether and shake well for 30 minutes, using short perpendicular strokes. Allow to settle and pipette out 25 cc. of clear ether solution. Evaporate in a weighed glass dish, dry the residue at 100° and weigh. In one experiment the weight of quinine was 0.9546 instead of 1 gram.

Caffeine was estimated in the same way, except that this alkaloid was extracted from the alkaline solution with chloroform after decomposition of potassium bismuthous iodide precipitate with alkaline hydroxide and carbonate. The weight of caffeine, melting at 231°, was 0.9546 instead of 1 gram.

The precipitate obtained by adding potassium bismuthous iodide to a solution of antipyrine in sulphuric acid (10 per cent. H_2SO_4) is not decomposed as easily as are those of quinine and caffeine. The precipitate from 2 grams of antipyrine must be shaken for 1 hour with 20 grams of sodium carbonate and 60 cc. of 10 per cent. sodium hydroxide solution. Antipyrine must be extracted with chloroform. The weight of antipyrine was 0.9273 instead of 1 gram.

Notes.—Potassium bismuthous iodide precipitates fixed and volatile alkaloids but not ammonium salts. If estimation of volatile bases is unnecessary, as in the examination of belladonna extract, evaporate 50 cc. of ether extract (see above) upon the water-bath. Warm the residue and in a few minutes the strong narcotic odor of volatile bases will disappear. Dissolve the residue in a little acid-free alcohol and dilute with ether. Before using a flask for titrations, carefully test beforehand for alkalinity. If a positive test is obtained, alkalinity must be removed. An odor like iodoform, probably due to the action of sodium hypoiodite upon the alkaloid, has been observed when sodium hydroxide solution acts upon potassium bismuthous iodide precipitates. Addition of sodium sulphite may prevent this action. After addition of sodium chloride, ether takes up the alkaloid more readily. But vigorous shaking is always needed to cause complete transfer of alkaloid to ether.

[1] According to investigations of D. Jonescu.

Estimation of Alkaloids in Ipecac and Pharmaceutical Preparations

1. Ipecac Root.—The German Pharmacopoeia prescribes 1.99 per cent. of alkaloids and calculates emetine as $C_{30}H_{44}N_2O_4$, mol. wt. 496.37. Emetine is a diacid base, its equivalent weight being 248.2. Add a mixture of 90 grams of ether and 30 grams of chloroform to 12 grams of finely powdered ipecac in a 180 cc. Erlenmeyer flask. Shake vigorously and add 5 grams of sodium carbonate solution (1 part of $Na_2CO_3.10H_2O$ and 2 parts of water) and 5 cc. of water. Shake frequently and allow the mixture to stand for 3 hours. When the chloroform-ether mixture is perfectly clear, filter 60 grams (= 6 grams of ipecac) through a well-covered dry paper into a small flask and distil the liquid. Warm the residue with 10 cc. of dilute hydrochloric acid (1 + 99) and filter the solution through a small paper moistened with water into a separating funnel (I). In the same manner make two more extractions of the residue with 5 cc. portions of dilute hydrochloric acid (1 + 99). Pass these extracts through the same paper and wash flask and paper well with water. Add to the combined hydrochloric acid extracts 5 cc. of chloroform and sodium carbonate solution until alkaline. Then shake the mixture vigorously for 2 minutes. As soon as the chloroform extract is perfectly clear, run into a separating funnel (II) and make three more extractions in the same manner with 5 cc. portions of chloroform. Add to the combined chloroform extracts 10 cc. of 0.1 n-hydrochloric acid and enough ether to float the chloroform-ether mixture upon the acid. Then shake vigorously for 2 minutes. As soon as the acid solution is perfectly clear, pass through a paper moistened with water into a 100 cc. graduated flask. Make three more extractions of the chloroform-ether mixture with 10 cc. portions of water, each time shaking for 2 minutes. Pass these extracts through the same paper, wash with water and dilute the total solution to 100 cc. with water. Measure into a flask 50 cc. of this solution (= 3 grams of ipecac), add about 50 cc. of water, and freshly prepared solution of a small particle[1] of haematoxylin in 1 cc. of alcohol. Shake and run in 0.1 n-sodium hydroxide solution until the mixture has a strong yellow color, changing to bluish violet on vigorous agitation. According to the German Pharmacopoeia this should require at most 2.6 cc. of 0.1 n-sodium hydroxide solution.

Calculation.—Alkaloids from 3 grams of ipecac are dissolved in 5 cc. of 0.1 n-hydrochloric acid. If titration of these alkaloids has required 2.6 cc. 0.1 n-sodium hydroxide solution, then $5 - 2.6 = 2.4$ cc. of 0.1 n-hydrochloric acid were combined with alkaloids. Since the equivalent weight of emetine is half its molecular weight = 248.2, 1000 cc. of 0.1 n-hydrochloric acid will combine with 24.82 grams and 1 cc. with 0.02482 gram of alkaloids. So 2.4 cc. correspond to $2.4 \times 0.02482 = 0.06$ gram of alkaloid (in round numbers), or a minimal content of 1.99 per cent. of alkaloids.

2. Tincture of Ipecac.—The tincture should contain at least 0.194 per cent. of alkaloids, taking emetine as $C_{30}H_{44}N_2O_4$ with the equivalent weight 248.2 (see above). Evaporate[2] 50 grams of tincture of ipecac in a weighed dish to 10 grams. Transfer this residue to an Erlenmeyer flask, rinsing with 5 grams of absolute alcohol. Add 20 grams of chloroform and 50 grams of ether, shake vigorously,

[1] It is preferable to dissolve several particles of haematoxylin in 1 cc. of alcohol.

[2] Evaporate the tincture upon a water-bath.

and add 5 cc. of sodium carbonate solution already used in rinsing the dish. Shake frequently and allow to stand for 1 hour. As soon as the chloroform-ether mixture is perfectly clear, pass 50 grams (= 33.33 grams of tincture of ipecac) through a well-covered dry paper into a weighed flask, and distil about two-thirds of the liquid. Transfer the cold residue to a separating funnel (I), rinse the flask three times with 5 cc. portions of a mixture of 2 parts of chloroform and 5 parts of ether. Also rinse once with 10 cc. of dilute hydrochloric acid (1 + 99). Pour these liquids into the separating funnel, add enough ether to float the chloroform-ether mixture upon the acid, and shake vigorously for 2 minutes. As soon as the hydrochloric acid solution is perfectly clear, run into a separating funnel (II) and make two more extractions in the same manner with 5 cc. portions of dilute hydrochloric acid (1 + 99) already used in rinsing the flask.

Add to the combined acid extracts 5 cc. of chloroform, then sodium carbonate solution until alkaline, and shake the mixture vigorously for 2 minutes. When separation is complete, run the chloroform extract into a separating funnel (III) and make three more extractions in the same manner with 5 cc. portions of chloroform. Add to the combined chloroform extracts 50 cc. of 0.01 n-hydrochloric acid and enough ether to float the chloroform-ether mixture upon the acid. Shake vigorously for 2 minutes and, when separation is complete, pass the acid solution through a small paper moistened with water into a 500 cc. flask of white glass. Make three more extractions of the chloroform-ether mixture with 10 cc. portions of water, shaking each time for 2 minutes. Pass these extracts through the same paper, wash with water, and dilute the combined extracts to about 100 cc. with water. Then add freshly prepared solution of a few particles of haematoxylin in 1 cc. of alcohol and 50 cc. of water. Shake gently and add 0.1 n-sodium hydroxide solution until the mixture has a strong yellow color, quickly changing to bluish violet upon vigorous agitation. At most 2.4 cc. of 0.1 n-sodium hydroxide solution should be sufficient.

Calculation.—Alkaloids from 33.33 grams of tincture of ipecac are dissolved in 50 cc. of 0.01 n-hydrochloric acid = 5 cc. 0.1 n-hydrochloric acid. At most 2.4 cc. of 0.1 n-sodium hydroxide solution should be used in titrating excess of hydrochloric acid. So 5 — 2.4 = 2.6 cc. of 0.1 n-hydrochloric acid are neutralized by alkaloids. This quantity of acid will neutralize 2.6 × 0.02482 = 0.0646 gram of alkaloids, corresponding to a minimal alkaloid-content in tincture of ipecac of 3 × 0.0646 = 0.194 per cent. (in round numbers).

Estimation of Cantharidin in Spanish Flies

(See page 348 for a fuller account of Cantharidin)

The German Pharmacopoeia requires a minimal content of 0.8 per cent. of cantharidin in Spanish flies. Place in an Erlenmeyer flask 15 grams of Spanish flies ground mediumly fine and add 150 grams of chloroform and 2 grams of hydrochloric acid (sp. gr. 1.124 = 25 per cent. HCl). Shake the mixture frequently during 24 hours. Then pour 102 grams of chloroform solution (= 10 grams of Spanish flies) through a well-covered dry filter (12 cm. in diameter), collecting the filtrate in a small flask accurately weighed upon an analytical balance. Distil chloroform completely with gentle heat and add 10 cc. of petroleum ether to the cold residue. Stopper the flask and allow the mixture

to stand for 12 hours with occasional agitation. Weigh a paper (5 cm. in diameter) dried at 100°, moisten with petroleum ether, and filter the liquid. Make four more extractions of undissolved residue with 5 cc. portions of petroleum ether, shaking each time. Pass these extractions through the same paper, disregarding crystals adhering to the sides of the flask. Having washed the rim of the filter by dropping 5 cc. of petroleum ether, dry flask and paper at 30–40°. Then wash filter and flask with a little water, containing a drop of ammonium carbonate solution to every 10 cc., until this solvent has scarcely any color. Finally wash once with 5 cc. of water. Then dry flask and paper first at 40–50°, place paper and contents in the flask, and dry at 100° to constant weight. Should cantharidin thus obtained not be well crystallized but resinous or dark in color, repeatedly extract with hot acetone, pass the solution through a small paper into an accurately weighed flask, and dry at 100° to constant weight. According to the German Pharmacopoeia, the weight of crystalline residue must be at least 0.08 gram, corresponding to a minimal content of 0.8 per cent. of cantharidin.

Notes.—Spanish flies contain cantharidin partly free and partly as an alkali salt of cantharidic acid. Hydrochloric acid sets cantharidic acid free and the latter at once passes into the lactone cantharidin, its internal anhydride. Consequently hydrochloric acid is essential to the determination of that cantharidin present in Spanish flies as cantharidate. Chloroform not only dissolves cantharidin but much fatty substance. To isolate pure cantharidin from these impurities, distil the chloroform and allow the residue to stand for 12 hours in the cold with petroleum benzine. Fat readily dissolves but cantharidin is practically insoluble in this solvent. With sufficient care, white crystalline cantharidin may be obtained. If only free cantharidin is to be determined in powdered Spanish flies, it should be extracted with chloroform, as directed above, without addition of hydrochloric acid. In one experiment 1.06 per cent. of total cantharidin was obtained, of which 0.72 per cent. was free and 0.34 per cent. combined with alkalies. Dietrich in a sample of powdered Spanish flies found only 0.3 per cent. of free cantharidin.

Estimation of Cinchona Alkaloids in Cinchona Barks and Preparations

Officinal cinchona barks should contain at least 6.5 per cent. of alkaloids having the composition $C_{20}H_{24}N_2O_2$ (Quinine) and $C_{19}H_{22}N_2O$ (Cinchonine).

1. **Cinchona Bark.**—Add 30 grams of chloroform and 30 grams of ether to 12 grams of finely powdered cinchona bark in an Erlenmeyer flask. Shake vigorously and add 5 grams of sodium hydroxide solution[1] and 5 cc. of water. Allow the mixture to stand 3 hours, shaking vigorously at frequent intervals. Then add 60 grams of ether and shake vigorously. When perfectly clear, pass 80 grams of chloroform-ether mixture (= 8 grams of cinchona bark) through a well-covered dry filter into a small flask and distil two-thirds of the solvent. Transfer the cold residue to a separating funnel (I), rinse the flask three times with 5 cc. portions of a mixture of 2 parts of chloroform and 5 parts of ether, and once with 20 cc. of dilute hydrochloric acid (1 + 99). Pour these washings

[1] Use officinal sodium hydroxide solution having the sp. gr. 1.168–1.172 and containing approximately 15 per cent. of NaOH.

into the separating funnel, add enough ether to float the chloroform-ether mixture upon the acid, and shake the mixture vigorously for 2 minutes. When the liquids are perfectly clear, run the acid solution into a separating funnel (II), and make two more extractions in the same manner with 5 cc. portions of dilute hydrochloric acid (1 + 99) already used in rinsing the flask. Add to the combined hydrochloric acid extracts 5 cc. of chloroform,[1] then sodium carbonate solution until alkaline, and shake the mixture vigorously for 2 minutes. When the liquids have separated perfectly clear, run the chloroform extract into a separating funnel (III), and make three more extractions in the same manner with 5 cc. portions of chloroform. Add to the combined chloroform extracts 25 cc. of 0.1 n-hydrochloric acid, then enough ether to float the chloroform-ether mixture upon the acid, and shake vigorously for 2 minutes. When the liquids are perfectly clear, pass the acid solution through a small paper moistened with water into a 100 cc. graduated flask. Make three more extractions of the chloroform-ether mixture with 10 cc. portions of water, shaking each time for 2 minutes. Pass these extracts through the same paper, wash with water, and dilute the total liquid to 100 cc. with water. Measure into a flask 50 cc. of this solution (= 4 grams of cinchona bark), add about 50 cc. of water, and freshly prepared solution of a small particle of haematoxylin in 1 cc. of alcohol. Shake gently and run in enough 0.1 n-sodium hydroxide solution to give the mixture a strong yellow color, quickly changing to bluish violet upon vigorous agitation.

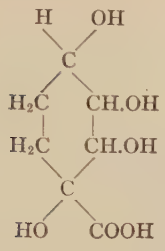
Officinal cinchona bark, should require at most 4.1 cc. of 0.1 n-sodium hydroxide solution. So 8.4 cc. at least of 0.1 n-hydrochloric acid are used to saturate alkaloids present, corresponding to a minimal content of 6.5 per cent. of alkaloids.

Notes and Calculations.—The isomeric alkaloids, quinine and quinidine ($C_{20}H_{24}N_2O_2$), and also the isomeric bases, cinchonine and cinchonidine ($C_{19}H_{22}N_2O$), are the most important alkaloids in cinchona bark. They are present in all true cinchona barks as salts of quinic acid ($C_{17}H_{12}O_6$) and quino-tannic acid.

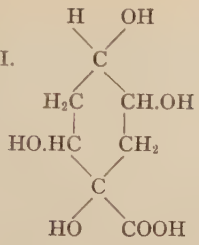
Quinic Acid.—Cinchona bark contains 5–8 per cent. of quinic acid which is widespread in the plant kingdom. This monobasic, pentatomic acid ($C_6H_7(OH)_4.COOH$) is a hexahydro-tetroxy-benzoic acid. Crystallizing in large monoclinic prisms (Mpt. 162°), this acid is readily soluble in water but difficultly in alcohol. Its solutions are laevo-rotatory: $[\alpha]_D = -44°$. Quinic acid contains four hydroxyl groups, since it forms a tetra-acetyl and a tetra-benzoyl derivative. At 220° it passes into an inner anhydride (lactone) known as quinid which with milk of lime forms the calcium salt of optically inactive quinic acid. Heated with hydriodic acid, quinic acid is converted into benzoic acid. Thus its relationship to the latter is clearly established. Added to a solution of quinic acid acidified with acetic acid, neutral lead acetate produces no precipitate, whereas basic lead acetate does. This behavior may be used to isolate quinic acid from plant extracts. The structure of quinic acid is expressed by one of the two following formulae, I or II:

[1] Take 10–20 cc. of chloroform, because then an emulsion is not so easily formed.

I.



II.



Addition of sodium hydroxide solution to cinchona powder sets the alkaloids free from their salts:

$$\begin{matrix} C_{20}H_{24}N_2O_2 \\ | \\ C_6H_7(OH)_4COOH \end{matrix} + NaOH = C_{20}H_{24}N_2O_2 + H_2O + C_6H_7(OH)_4COONa$$

Quinine quinate — Free quinine — Sodium quinate

Quinine dissolves in chloroform-ether mixture and is again extracted from that solvent by hydrochloric acid. The first separation of cinchona alkaloids from this hydrochloric acid extract is made by sodium carbonate:

$$2C_{20}H_{24}O_2N_2.HCl + Na_2CO_3 = 2C_{20}H_{24}O_2N_2 + H_2O + CO_2 + 2NaCl$$

Very dilute acid, 0.1 n-HCl, is used in titrating cinchona alkaloids. In presence of this acid these alkaloids act like monacid bases, quinine dissolving as $C_{20}H_{24}O_2N_2.HCl$ and cinchonine as $C_{19}H_{22}ON_2.HCl$. Quinine dihydrochloride is formed when hydrochloric acid gas is passed over quinine, or when officinal quinine monohydrochloride is dissolved in fuming hydrochloric acid and the resulting solution evaporated by gentle heat.

So the equivalent weights of quinine and cinchonine in these titrations are the same as their molecular weights. Since it is approximately in agreement with the relations, as they exist in cinchona bark, the arithmetical mean of the equivalent weights of quinine (324) and cinchonine (294), that is, (324 + 294), divided by 2 = 309, may be taken as the basis of the calculation. 1000 cc. of 0.1 n-HCl saturate 30.9 grams of cinchona alkaloids; 1 cc. of 0.1 n-acid = 0.0309 gram of mixed alkaloids.

Example.—Alkaloids set free by sodium carbonate and dissolved by chloroform are extracted from the chloroform solution by 25 cc. of 0.1 n-HCl and this solution is diluted with water to 100 cc. In measuring excess of 0.1 n-HCl in 50 cc. of this HCl solution, 2.6 cc. of 0.1 n-NaOH are required. 12.5 − 2.6 = 9.9 cc. of 0.1 n-HCl were therefore combined with alkaloids from 4 grams of cinchona bark. So these 4 grams of bark contain 9.9 × 0.0309 = 0.30591 gram of alkaloids and the alkaloidal content of cinchona bark examined is 25 × 0.30591 = 7.04 per cent.

Notes.—The German Pharmacopoeia requires that the chloroform-ether solution of free cinchona alkaloids be tested at once. This solution should not be exposed for any length of time to direct sunlight, otherwise oxidation of chloroform may give rise to free hydrochloric acid:

$$CHCl_3 + O = COCl_2 + HCl$$

This acid obviously will then neutralize part of the free alkaloids. Neutralization of 0.25 gram of quinine requires the decomposition of only 0.05 gram of chloroform. In fact Panchaud[1] found that such chloroform solutions of cinchona alkaloids after standing 12 hours yield only 80 per cent. of total alkaloids originally present.

Haematoxylin.—This compound, $C_{16}H_{14}O_6.3H_2O$, occurs in logwood, the heart-wood of Haematoxylon campechianum freed from bark and sap-wood. Usually it crystallizes in colorless, shining, quadratic prisms containing 3 molecules of water, more rarely in rhombic crystals with 1 molecule of water. Only slightly soluble in cold water, it dissolves freely in boiling water, alcohol and ether. Exposed to light it gradually becomes reddish. Dissolved in aqueous ammonia, or in alkaline hydroxides or carbonates, it turns blue-violet in contact with air. Aqueous or alcoholic solution of haematoxylin is a sensitive reagent for substances having an alkaline reaction in aqueous solution. As an indicator in volumetric analysis its use is limited.

2. **Aqueous and Alcoholic Cinchona Extracts.**—Dissolve 3 grams of aqueous, or 2 grams of alcoholic cinchona extract in 5 cc. of water and 5 grams of absolute alcohol in an Erlenmeyer flask. Add 20 grams of chloroform, shake well, and then add 10 cc. of sodium carbonate solution.[2] Shake the mixture often and allow to stand for one hour. Then add 50 grams of ether, shake well, and, when the ether-chloroform mixture is perfectly clear, filter 50 grams (= 2 grams of aqueous = 1.33 gram of alcoholic cinchona extract) through a well-covered, dry paper into a small flask, and distil about two-thirds of the solvent. When the residue is cold, transfer to a separating funnel (I), rinse the flask with three 5 cc. portions of a mixture of 2 parts of chloroform and 5 parts of ether and finally with 10 cc. of dilute hydrochloric acid (1 + 99). Pour these rinsings into the separating funnel. Then add enough ether to float the chloroform-ether mixture upon the acid solution and shake the mixture well for 2 minutes. When the two liquids have separated perfectly clear, run the hydrochloric acid solution into a separating funnel (II), and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 99) previously used to rinse the flask.

Add to the combined hydrochloric acid extracts 5 cc. of chloroform and enough sodium carbonate solution to render alkaline. Shake the mixture well for 2 minutes. When the liquids have separated perfectly clear, run the chloroform extract into a separating funnel (III) and in the same manner make three more extractions with 5 cc. portions of chloroform. Add to the combined chloroform extracts 10 cc. of 0.1 n-HCl and enough ether to float the chloroform-ether mixture upon the acid solution. Shake the mixture well for 2 minutes. When the liquids have separated perfectly clear, filter the acid solution through a small paper wet with water into a 100 cc. graduated flask. Make three more extractions of the chloroform-ether mixture, each time shaking for 2 minutes with 10 cc. of water. Pass these extracts through the same paper, washing with enough water to bring the total volume of solution to 100 cc. Measure into a flask

[1] Panchaud: Schweizerische Wochenschr. f. Pharmazie, 44,580.

[2] According to the German Pharmacopoeia, this solution is prepared by dissolving 1 part of $Na_2CO_3.10H_2O$ in 2 parts of water.

50 cc. of this solution (= 1 gram of aqueous, or 0.67 gram of alcoholic cinchona extract), add 50 cc. of water and freshly prepared solution of a small particle of haematoxylin in 1 cc. of alcohol. Shake gently and run in enough 0.1 n-NaOH to give the mixture a strong yellow color, quickly changing to bluish violet after vigorous shaking. At most the end-reaction should require 3 cc. for aqueous and 2.4 cc. 0.1 n-NaOH for alcoholic cinchona extract.

Calculation.—The basis of calculation in these determinations of alkaloids in the two cinchona extracts, as in that of cinchona bark, is the arithmetical mean of the equivalent weights of quinine and cinchonine, namely, 309. The 50 cc. of hydrochloric acid solution of alkaloids is half of the 10 cc. originally taken, or 5 cc. of 0.1 n-HCl. Of these, 3 cc. are titrated back by 0.1 n-NaOH and so 2 cc. of alkaloids and 1 gram of aqueous extract require = 2 × 0.0309 = 0.0618 gram of alkaloids, corresponding to a minimal content of 6.18 per cent. of alkaloids. Similarly alkaloids in 0.67 gram of alcoholic cinchona extract should require 5 − 2.4 = 2.6 cc. of 0.1 n-HCl, which is 2.6 × 0.0309 = 0.08054 gram of alkaloids, corresponding to a minimal content of 12 per cent. of alkaloids in round numbers.

3. **Fluid Extract of Cinchona.**—In a weighed dish evaporate upon the water-bath 10 grams of fluid extract of cinchona to about 5 grams. Transfer the residue while warm to an Erlenmeyer flask, adding 5 grams of absolute alcohol previously used in small portions to rinse the dish. Then add to the mixture 25 grams of chloroform and 20 grams of ether. Shake well and add 6 cc. of sodium carbonate solution. Allow the mixture to stand for one hour and shake well from time to time. Now add 50 grams of ether and shake well. Filter 80 grams of the perfectly clear chloroform-ether mixture (= 8 grams of fluid extract of cinchona) through a well-covered, dry paper into a weighed flask and distil about half of the solvent. Transfer the cold residue to a separating funnel (I), rinsing the flask three times with 5 cc. portions of ether and once with dilute hydrochloric acid (1 + 99). Pour these rinsings into the separating funnel and shake well for two minutes. Run the perfectly clear hydrochloric acid solution into a separating funnel (II) and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 99) previously used to rinse the flask. From this point on, beginning with the words "Add to the combined hydrochloric acid extracts 5 cc. of chloroform and enough sodium carbonate solution to render alkaline . . . " the procedure is the same as for aqueous cinchona extract.[1]

Calculation.—The end-point in titration should require at most, according to the German Pharmacopoeia, 5.4 cc. of 0.1 n-NaOH. So 10 − 5.4 = 4.6 cc. of 0.1 n-HCl should neutralize alkaloids in 4 grams of fluid extract of cinchona. This is 4.6 × 0.0309 = 0.1422 gram of alkaloids, corresponding to a minimal content of 3.5 per cent. Similarly 5 − 2.4 = 2.6 cc. of n-HCl (= 2.6 × 0.0309 gram = 0.08054 gram of alkaloids) neutralize alkaloids in 0.67 gram of alcoholic

[1] "In case of fluid extract of cinchona, add 20 cc. of 0.1 n-hydrochloric acid to the combined chloroform extracts . . . " and not 10 cc. as directed for aqueous and alcoholic extracts. Moreover the 50 cc. portions, in which at the end excess of 0.1 n-hydrochloric acid is determined by titration, contain alkaloids from 4 grams of fluid extract of cinchona.

extract of cinchona. This corresponds to a minimal content of 12 per cent. of alkaloids in round numbers.

4. **Tincture of Cinchona and Compound Tincture of Cinchona.**—In a weighed dish evaporate 50 grams of tincture of cinchona, or the same weight of compound tincture, to 10 grams. Transfer the residue to an Erlenmeyer flask, rinsing the dish with 5 grams of absolute alcohol. Add 20 grams of chloroform, shake well, and add 5 cc. of sodium carbonate solution previously used to rinse the dish. Allow the mixture to stand for one hour, shaking well from time to time. Then add 50 grams of ether, shake well, filter 50 grams of perfectly clear chloroform-ether mixture (= 33.33 grams of the simple or compound tincture of cinchona) through a well-covered, dry paper into a small flask, and distil the solvent. Warm the residue with 20 cc. of dilute hydrochloric acid (1 + 99), and filter the solution through a small paper wet with water into a separating funnel (I). In the same manner make two more extractions of the residue with 5 cc. portions of dilute hydrochloric acid (1 + 99), and pass these extracts through the same paper, washing flask and filter well with water. Then add to the combined hydrochloric acid extracts 10 cc. of chloroform and enough officinal sodium hydroxide solution to render alkaline. At once shake the mixture well for two minutes. Run the chloroform when perfectly clear (hastened by gentle warming) into a separating funnel (II), and in the same manner make three more extractions with 5 cc. portions of chloroform. Add to the combined chloroform extracts 20 cc. of 0.1 n-HCl and enough ether to float the chloroform-ether mixture upon the acid solution. Shake well for two minutes. Filter the perfectly clear acid aqueous solution through a small paper wet with water into a 100 cc. graduated flask. Make three more extractions of chloroform-ether mixture, shaking each time for two minutes with 10 cc. portions of water, pass these extracts through the same paper, wash with water, and dilute the total solution with water to 100 cc., having first added about 0.01 gram of freshly ignited animal charcoal. Filter the completely decolorized solution through a small dry paper and measure 50 cc. of filtrate = 16.67 grams of simple or compound tincture of cinchona. Add 50 cc. of water and freshly prepared solution of a small particle of haematoxylin in 1 cc. of alcohol. Shake gently and run in enough 0.1 n-NaOH to produce a strong yellow color, changing to reddish violet after vigorous shaking. In case of tincture of cinchona 6 cc. of 0.1 n-NaOH should produce this coloration. So the alkaloids in 16.67 grams of tincture have taken at least 4 cc. of 0.1 n-HCl. This is $4 \times 0.0309 = 0.1236$ gram of alkaloids, corresponding to a minimal content of 0.74 per cent. of alkaloids. For appearance of the reddish violet coloration, compound tincture of cinchona should require at most 8 cc. of 0.1 n-NaOH. So neutralization of the alkaloids in 16.67 grams of tincture takes at least 2 cc. of 0.1 n-HCl. This is $2 \times 0.0309 = 0.0618$ gram of alkaloids, corresponding to a minimal content of 0.37 per cent. of alkaloids.

Estimation of Alkaloids in Pomegranate Bark

(See pages 376–380)

Officinal pomegranate bark should contain at least 0.4 per cent. of alkaloids. An average equivalent weight of 148 is taken.

Pour 120 grams of ether upon 12 grams of finely ground pomegranate bark in a 180 cc. Erlenmeyer flask. Shake well and add 10 cc. of a mixture of 1 part of

sodium hydroxide solution and 1 part of water. Allow the mixture to stand for three hours, shaking well from time to time. Filter 80 grams of perfectly clear ether solution (= 8 grams of pomegranate bark) through a well-covered, dry paper into a small flask and distil about half the ether at as low a temperature as possible. Transfer the cold residue to a separating funnel (I), rinsing the flask three times with 5 cc. portions of ether and once with 10 cc. of dilute hydrochloric acid (1 + 99). Pour these washings into the separating funnel and shake the mixture well for two minutes. Run the perfectly clear hydrochloric acid solution into a separating funnel (II) and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 99) previously used to rinse the flask.

Add to the combined hydrochloric acid extracts 5 cc. of chloroform and enough sodium carbonate solution to render alkaline. At once shake the mixture well for two minutes. Run the perfectly clear chloroform extract into a separating funnel (III) and in the same manner make three more extractions with 5 cc. portions of chloroform. Add to the combined chloroform extracts 40 cc. of 0.01 n-HCl, enough ether to float the chloroform-ether mixture upon the acid solution, and shake well for two minutes. Filter the perfectly clear acid solution through a small paper wet with water into a 200 cc. white glass flask. Make three more extractions of the chloroform-ether mixture, each time shaking for two minutes with 10 cc. portions of water. Pass these extracts through the same paper, washing with water and bringing the total volume to about 100 cc.

Add enough ether to form a layer 1 cm. deep and 10 drops of iodeosine solution. Run in 0.01 n-NaOH, shaking the mixture well after each addition, until the lower aqueous layer has a pale red color. This should require at most 18 cc. of 0.01 n-NaOH. So the alkaloids have neutralized at least 22 cc. of 0.01 n-HCl, corresponding to a minimal content of 0.4 per cent. of alkaloids in pomegranate bark. With iodeosine as indicator, 1 cc. of 0.01 n-HCl = 0.00148 gram of alkaloids in pomegranate bark (German Pharmacopoeia).

Estimation of Hydrastine[1] in Hydrastis Root and Fluid Extract

The root of Hydrastis canadensis contains the alkaloids, berberine, canadine, and 2.5 per cent. of hydrastine or more. The German Pharmacopoeia requires a minimal content of 2.5 per cent. of hydrastine, $C_{21}H_{21}O_6N$.

1. Hydrastis Root.—Put in an Erlenmeyer flask 6 grams of hydrastis root ground mediumly fine and add 60 grams of ether. Shake well and add 10 cc. of ammonia. Allow the mixture to stand for three hours, shaking well from time to time. Filter 40 grams of perfectly clear ether solution (= 4 grams of hydrastis root) through a well-covered, dry paper into a small flask and distil the ether. Warm the residue with 10 cc. of dilute hydrochloric acid (1 + 99) and filter the solution through a small paper wet with water into a separating funnel. Rinse the flask twice with 5 cc. portions of dilute hydrochloric acid (1 + 99), pass these washings through the same paper into the separating funnel, and wash with a little water. Add to the combined hydrochloric acid extracts 40 cc. of ether, shake the mixture well, add ammonia until alkaline, and at once shake the mixture well for two minutes. Run out the perfectly clear aqueous solution. Shake

[1] See pages 205 to 209.

around the ether solution remaining in the separating funnel and when clear measure 30 cc. = 3 grams of hydrastis root. Evaporate ether at moderate heat in a light weighed flask and dry the residue at 100° to constant weight. The residue should weigh at least 0.075 gram, corresponding to a minimal content of 2.5 per cent. of hydrastine in hydrastis root.

2. Fluid Extract of Hydrastis.—Officinal fluid extract of hydrastis must contain at least 2.2 per cent. of hydrastine. Add water to 10 grams of fluid extract of hydrastis in a weighed dish and evaporate upon the water-bath to about 8 grams. Add 1.5 cc. of dilute hydrochloric acid (sp. gr. 1.062 = 12.5 per cent. HCl) and transfer the mixture to a weighed flask. Carefully rinse the dish with 1.5 cc. portions of water until the weight of combined liquids is 20 grams. Add 1 gram of talcum, shake well, and filter through a dry paper (8 cm. diameter) into a dry flask. Put 10 grams of this filtrate (= 5 grams of fluid extract of hydrastis) into a 100 cc. Erlenmeyer flask, adding 4 cc. of ammonia and 30 cc. of ether. Shake the mixture well for several minutes. Then add 30 cc. of petroleum benzine and shake again for several minutes. Add 1.5 grams of powdered tragacanth and shake well until the ether layer is perfectly clear. Filter the latter through a well-covered, dry paper into a dry flask and at once transfer 40 cc. of filtrate (= 3.33 grams of fluid extract of hydrastis) to an accurately weighed flask.[1] Evaporate ether spontaneously at about 30°, completely dry the residue at 100°, cool in desiccator, and weigh. The residue should weigh at least 0.073 gram, corresponding to a minimal content of 2.2 per cent. of hydrastine.

Notes.—Hydrastis root contains about 4 per cent. of berberine which unlike hydrastine is not precipitated from solutions of its salts by ammonia. So if the hydrochloric acid solution is rendered alkaline with ammonia and extracted with ether, the latter dissolves only hydrastine. Upon this difference in behavior the German Pharmacopoeia bases the separation of hydrastine from berberine.

Estimation of Hyoscyamine in Belladonna Leaf, Hyoscyamus Leaf, and Officinal Extracts

The main portion of alkaloids contained in leaves (up to 0.4 per cent.), fruit (unripe, dried fruit contains up to 0.8 per cent.; ripe, dried fruit up to 0.4 per cent. of alkaloid), seeds (up to 0.8 per cent.), and root (up to 0.5 per cent.) of Atropa Belladonna, as well as in different parts of Datura Stramonium, is hyoscyamine and not atropine as formerly supposed. Atropine obtained in isolating alkaloids from various parts of these plants is in fact formed by molecular rearrangement, that is, by racemization of hyoscyamine. Together with scopolamine, and possibly also with atropine, hyoscyamine also occurs in seeds and leaves (up to 0.3 per cent.) of Hyoscyamus niger and also in the root of Scopolia atropoides.

1. Belladonna Leaf (Deadly Nightshade) and Hyoscyamus Leaf (Henbane).—Add 120 grams of ether to 20 grams of finely ground deadly nightshade, or henbane leaves, in an Erlenmeyer flask. Having shaken well, add 5 grams of sodium hydroxide solution and 5 cc. of water. Allow the mixture to stand for one hour, shaking well from time to time. Filter 60 grams of perfectly clear ether solution (= 10 grams of deadly nightshade, or 10 grams of henbane), through a well-cov-

[1] Weigh accurately upon an analytical balance the flask itself and then with hydrastine obtained.

ered, dry paper into a small flask and distil about two-thirds of the ether.(*) Transfer the cold residue to a separating funnel (I), rinsing the flask three times with 5 cc. portions of ether and once with 10 cc. of hydrochloric acid (1 + 49). Pour these liquids into the separating funnel and shake well for two minutes. Run the perfectly clear hydrochloric acid solution into separating funnel (II) and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 49) previously used to rinse the flask. Add to the combined hydrochloric acid extracts 5 cc. of chloroform, enough sodium carbonate solution to render alkaline, and at once shake the mixture well for two minutes. Run the perfectly clear chloroform extract into a separting funnel (III) and in the same manner make three more extractions with 5 cc. portions of chloroform. Add to the combined chloroform extracts 20 cc. of 0.01 n-HCl[1] and enough ether to float the chloroform-ether mixture upon the acid. Shake well for two minutes. Filter the perfectly clear acid solution through a small paper wet with water into a 200 cc. white glass flask. Make three more extractions of chloroform-ether mixture with 10 cc. portions of water, each time shaking for two minutes. Pass these extracts through the same paper, wash with water, and bring the volume of the liquid to about 100 cc. Add enough ether to form a layer 1 cm. deep and 10 drops of iodeosine solution. Run in 0.01 n-NaOH, shaking well after each addition, until the lower aqueous layer has a pale red color.

Deadly nightshade leaves should require at most 9.6 cc. of 0.01 n-NaOH. So the alkaloids present have neutralized at least 10.4 cc. of 0.01 n-HCl.

Henbane leaves should require at most 7.6 cc. of 0.01 n-NaOH. So the alkaloids present have neutralized at least 2.4 cc. of 0.01 n-HCl.

Calculation.—The equivalent weight of the monacid base, hyoscyamine, is the same as its molecular weight, $C_{17}H_{23}O_3N = 289$. So 1000 cc. of 0.01 n-HCl neutralize 2.89 grams of hyoscyamine and 1 cc. neutralizes 0.00289 gram. Hence 10 grams of deadly nightshade leaves contain $10.4 \times 0.00289 = 0.03$ gram of hyoscyamine, corresponding to a minimal content of 0.3 per cent. of alkaloid. Similarly 10 grams of henbane leaves should contain at least $2.4 \times 0.00289 = 0.007$ gram of hyoscyamine, corresponding to a minimal content of 0.07 per cent. of alkaloid.

2. Extract of Belladonna (Deadly Nightshade) and Extract of Hyoscyamus (Henbane).—Dissolve 3 grams of extract of deadly nightshade, or henbane, in 5 cc. of water and 5 grams of absolute alcohol in an Erlenmeyer flask. Add 70 grams of ether, shake well, and then add 5 cc. of sodium carbonate solution. Allow the mixture to stand for one hour, shaking well from time to time. Filter 50 grams of perfectly clear ether solution (= 2 grams of extract) through a well-covered, dry paper into a small flask and distil about two-thirds of the ether. From the place marked with an asterisk(*) the procedure is the same as that already described for deadly nightshade, or henbane, leaves (see above) except that in the determination of hyoscyamine in henbane the directions should read "Add to the combined chloroform extracts 10 cc. of 0.01 n-HCl . . . " instead of 20 cc. (see above).

Calculation. Extract of Deadly Nightshade.—Titration of excess of acid should require 9.6 cc. of 0.01 n-NaOH. So 20 − 9.6 = 10.4 cc. of 0.01 n-HCl are

[1] In the examination of henbane leaves take only 10 cc. of 0.01 n-hydrochloric acid.

used to neutralize hyoscyamine in 2 grams of extract which contain 10.4 × 0.00289 = 0.03 gram of hyoscyamine, corresponding to 1.5 per cent. of alkaloid.

Extract of Henbane.—Titration of excess of acid should require 6.5 cc. of 0.01 n-NaOH. So 10 − 6.5 = 3.5 cc. of 0.01 n-HCl are used to neutralize alkaloid in 2 grams of extract which contain 3.5 × 0.00289 = 0.0101 gram of alkaloid, corresponding to 0.505 per cent. of hyoscyamine.

Estimation of Morphine in Opium and Pharmaceutical Preparations

1. Powdered Opium.—Triturate 7 grams of powdered opium with 7 cc. of water, washing the mixture with water into a small weighed flask and bringing the weight to 63 grams by further addition of water. Shake often and allow the mixture to stand for one hour. Then filter through a dry 10 cm. creased paper. Add 2 cc. of a mixture of 17 grams of ammonia[1] and 83 cc. of water to 42 grams of filtrate = 4.88 grams of powdered opium. Avoid vigorous shaking. Filter the mixture at once through a dry 10 cm. creased paper into a small weighed flask. Add 10 cc. of acetic ether, and also 5 cc. of the mixture of 17 grams of ammonia and 83 cc. of water, to 36 grams of filtrate, weighed in this small flask, (= 4 grams of powdered opium) while gently shaking. Stopper the flask and shake contents for ten minutes. Add 20 cc. more of acetic ether and allow the mixture to stand for 15 minutes, shaking gently from time to time. Then pour the layer of acetic ether as completely as possible upon an 8 cm. smooth paper. Add to the water solution in the flask 10 cc. more of acetic ether, rotating the mixture for a few minutes and again pouring the acetic ether layer upon the paper. When the acetic ether has passed through, pour the water solution upon the paper, disregarding crystals adhering to the flask. Wash both paper and flask with three 5 cc. portions of water saturated with acetic ether. As soon as the flask is well-drained and there is no further dropping from the funnel, dry both at 100°. Then dissolve crystals of morphine in 25 cc. of 0.1 n-HCl and pour the solution into a 100 cc. graduated flask. Carefully wash paper, flask and stopper with water and finally dilute the solution with water to 100 cc. Measure into a 200 cc. white glass flask 50 cc. of this solution (= 2 grams of opium powder), add about 50 cc. of water, and enough ether to form a layer 1 cm. deep. Add 10 drops of iodeosine solution and run in 0.1 n-NaOH, shaking the mixture well after each addition, until the lower aqueous layer has a pale red color. The German Pharmacopoeia requires 5.5 cc. of 0.1 n-NaOH. So morphine present should neutralize 7 cc. of 0.1 n-HCl, corresponding to 10 per cent. of morphine. 1 cc. of 0.1 n-HCl = 0.02852 gram of morphine, with iodeosine as indicator.

2. Extract of Opium.—Dissolve 3 grams of extract of opium in 40 cc. of water and while shaking gently add 2 cc. of a mixture of 17 grams of officinal ammonia and 83 cc. of water. Filter at once through a 10 cm. creased paper. Transfer to a small flask 30 grams of filtrate = 2 grams of extract of opium. Gently rotate the flask and add 10 cc. of acetic ether. Add also 5 cc. of a mixture of 17 grams of ammonia and 83 cc. of water, stopper the flask, and shake well for ten minutes. Then add 20 cc. more of acetic ether and allow to stand for 15 minutes, shaking gently from time to time.(*) Pour the acetic ether layer as completely as possible upon an 8 cm. smooth paper. Add to the aqueous solution remaining in the

[1] By this is meant an aqueous solution containing 10 per cent. of NH_3.

flask 10 cc. more of acetic ether, rotate the mixture for a few minutes, and again pour the acetic ether layer upon the paper. When the acetic ether solution has passed through, pour the aqueous solution upon the paper, disregarding crystals adhering to the flask. Wash both paper and flask three times with 5 cc. portions of water saturated with acetic ether. Dry flask and paper at 100°. Dissolve crystals in 25 cc. of 0.1 n-HCl and pour the solution into a 100 cc. graduated flask. Carefully wash paper, flask and stopper with water and dilute the solution to 100 cc. Measure into a 200 cc. white glass flask 50 cc. of this solution (= 1 gram of extract of opium), adding about 50 cc. of water and enough acetic ether to form a layer 1 cm. deep. Add 10 drops of iodeosine solution and then run in 0.1 n-NaOH, shaking well after each addition, until the lower aqueous layer has a pale red color. To obtain morphine in 1 gram of extract of opium, multiply by 0.02852 the number of cc. of 0.1 n-HCl required to neutralize morphine.

In determining the strength of extract of opium said to contain 20 per cent. of morphine, excess of 0.1 n-HCl should require 5.5 cc. of 0.1 n-NaOH. So the morphine present has neutralized 7 cc. of 0.1 n-HCl.

3. Simple Tincture of Opium.—Evaporate 50 grams of simple tincture of opium to 15 grams in a weighed dish. Bring the weight to 38 grams with water and, while gently rotating the dish, add 2 cc. of a mixture of 17 grams of ammonia and 83 cc. of water. Filter the mixture at once through a dry 10 cm. creased paper into a small weighed flask. Add 10 cc. of acetic ether and 5 cc. of a mixture of 17 grams of ammonia and 83 cc. of water to 32 grams of the filtrate (= 40 grams of simple tincture of opium), while gently shaking. Stopper the flask and shake contents well for 10 minutes. Add 20 cc. more of acetic ether and allow to stand 15 minutes, shaking gently from time to time. From this point the procedure for tincture of opium is the same as for extract of opium. The place above marked with an asterisk (*) begins with the words "Pour the acetic ether layer . . . " The 50 cc. portion of solution, in which excess of 0.1 n-HCl is determined, contains morphine from 20 grams of simple tincture of opium. To obtain morphine in 20 grams of tincture of opium, multiply by 0.02852 the number of cc. of 0.1 n-HCl required to neutralize morphine.

In determining the strength of a standardized tincture of opium, excess of 0.1 n-HCl should require 5.5 cc. 0.1 n-NaOH. So morphine present has neutralized 7 cc. of 0.1 n-HCl, corresponding to 1 per cent. of alkaloid.

Notes.—Opium alkaloids are combined with meconic acid and to some extent also with sulphuric acid. Excess of ammonia sets morphine and most of the other alkaloids free from these salts:

$$(C_{17}H_{19}NO_3)_2 \cdot C_5HO_2(OH)(COOH)_2 + 2(H_4N)OH = 2C_{17}H_{19}NO_3 + C_5HO_2\begin{matrix}OH\\(COONH_4)_2\end{matrix} + 2H_2O$$

Morphine meconate — Morphine — Ammonium meconate

Acetic ether holds other opium alkaloids in solution but dissolves only a trace of crystallized morphine. The solubility of morphine in acetic ether is 1:1665.

The funnel and paper, containing free morphine washed with water containing acetic ether, is loosely covered with a small watch-glass to prevent loss of morphine from rapid evaporation of adherent acetic ether during drying in the steam-closet.

Morphine Meconate.—The compound $(C_{17}H_{19}O_3N)_2.C_7H_4O_7.5H_2O$, is a constituent of opium and crystallizes in whitish, star-shaped clusters of needles readily soluble in water. To prepare this compound, neutralize powdered free morphine base (2 mols.) with an aqueous solution of meconic acid (1 mol.) and evaporate the filtered solution at moderate heat to the point of crystallization.

Calculation.—Since morphine is a monacid base, its molecular and equivalent weight are the same = $C_{17}H_{19}O_3N$ = 285.2, and 285.2 grams of free morphine will neutralize 1 gram equivalent weight of a strong acid. So 1000 cc. of 0.1 n-HCl neutralize 28.52 grams and 1 cc. of 0.1 n-HCl neutralizes 0.02852 gram of $C_{17}H_{19}O_3N$.

The 50 cc. of the hydrochloric acid solution finally titrated contain morphine from 2 grams of opium and excess of 0.1 n-HCl. If 5.5 cc. of 0.1 NaOH were required to titrate excess of acid, morphine from 2 grams of opium must have combined with 12.5 − 5.5 = 7.0 cc. of 0.1 n-HCl. This volume of 0.1 n-acid corresponds to 7 × 0.02852 = 0.1997 gram of $C_{17}H_{19}O_3N$. So 100 grams of opium examined contain 50 × 0.1997 = 10.0 grams of morphine in round numbers.

Estimation of Strychnine and Brucine in Nux Vomica and Preparations

1. Nux Vomica.—Put in an Erlenmeyer flask 15 grams of nux vomica ground mediumly fine and add 50 grams of ether and 15 grams of chloroform. Shake well, adding 5 grams of sodium hydroxide solution[1] and 5 cc. of water. Allow the mixture to stand for 3 hours, shaking well from time to time. Then add 50 grams of ether, shake well, filter 100 grams of perfectly clear chloroform-ether solution (= 10 grams of nux vomica) through a well-covered, dry paper into a small flask, and distil about two-thirds of the solvent. Transfer the cold residue to a separating funnel (I), rinsing the flask three times with 5 cc. portions of a mixture of 2 parts of chloroform and 5 parts of ether and once with 10 cc. of dilute hydrochloric acid (1 + 99). Pour these liquids also into the separating funnel (I). Then add enough ether to float the chloroform-ether solution upon the acid and shake well for 2 minutes. Run the perfectly clear hydrochloric acid solution into another separating funnel (II) and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 99) previously used to rinse the flask.

Add to the combined hydrochloric acid extracts 5 cc. of chloroform, enough sodium carbonate solution to render alkaline, and at once shake the mixture well for 2 minutes. Run the perfectly clear chloroform extract into a separating funnel (III) and in the same manner make three more extractions with 5 cc. portions of chloroform. Add to the combined chloroform extracts 10 cc. of 0.1 n-HCl (= 100 cc. of 0.01 n-acid), enough ether to float the chloroform-ether mixture upon the acid, and shake well for 2 minutes. Filter the perfectly clear acid solution through a small paper wet with water into a 100 cc. graduated flask. Make

[1] Officinal sodium hydroxide solution contains approximately 15 per cent. of NaOH and has a sp. gr. of 1.168–1.172.

three more extractions of the chloroform-ether mixture, each time shaking for 2 minutes with a 10 cc. portion of water. Pass these extracts through the same paper, wash with water, and bring the total volume of liquid to 100 cc. with water. Measure into a 200 cc. white glass flask 50 cc. of this solution (= 5 grams of nux vomica), adding about 50 cc. of water and enough ether to form a layer about 1 cm. deep. Add 10 drops of iodeosine solution and 0.01 n-NaOH, shaking the mixture well after each addition, until the lower aqueous layer has a pale red color. This should require at most 15.6 cc. of 0.01 n-NaOH. So the alkaloids present have neutralized at least 34.4 cc. 0.01 n-HCl, corresponding to a minimal content of 2.5 per cent. of alkaloids. 1 cc. of 0.01 n-HCl = 0.00364 gram of strychnine and brucine in equal parts, with iodeosine as indicator. Strychnine, $C_{21}H_{22}N_2O_2$ (Mol. Wt. 334); brucine, $C_{23}H_{26}N_2O_4$ (Mol. Wt. 394).

2. Extract of Nux Vomica.—Dissolve with gentle heat in an Erlenmeyer flask 1.2 grams of extract of nux vomica in 5 cc. of water, 5 cc. of absolute alcohol, and 1 cc. of dilute sulphuric acid (1 + 4). Cool this solution, adding 20 grams of chloroform and, after shaking well, 2 cc. of sodium hydroxide solution and 5 cc. of sodium carbonate solution. Shake well from time to time and allow the mixture to stand for one hour (*). Then add 50 grams of ether, shake well, filter 50 grams of perfectly clear chloroform-ether solution (= 0.8 gram of extract of nux vomica) through a well-covered dry paper into a small flask, and distil about two-thirds of solvent. Transfer the cold residue to a separating funnel (I), rinsing the flask three times with 5 cc. portions of a mixture of 2 parts of chloroform and 5 parts of ether and once with dilute hydrochloric acid (1 + 99). Pour these liquids into the separating funnel, add enough ether to float the chloroform-ether mixture upon the acid, and shake well for 2 minutes. Run the perfectly clear hydrochloric acid solution into a separating funnel (II) and in the same manner make two more extractions with 5 cc. portions of dilute hydrochloric acid (1 + 99) previously used to rinse the flask.

Add to the combined hydrochloric acid extracts 5 cc. of chloroform, enough sodium carbonate solution to render alkaline, and at once shake the mixture well for 2 minutes. Run the perfectly clear chloroform extract into a separating funnel (III) and in the same manner make three more extractions with 5 cc. portions of chloroform. Add (**) to the combined chloroform extracts 50 cc. of 0.01 n-HCl, enough ether to float the chloroform-ether mixture upon the acid, and shake well for 2 minutes. Filter the perfectly clear acid solution through a small paper wet with water into a 200 cc. white glass flask. Make three more extractions of chloroform-ether mixture, shaking each time for 2 minutes with 10 cc. portions of water. Pass these aqueous extracts through the same paper, washing with water and bringing the total volume of the filtrate to about 100 cc. Add enough ether to form a layer 1 cm. deep, 10 drops of iodeosine solution, and 0.01 n-NaOH, shaking the mixture well after each addition, until the lower aqueous layer has a pale red color. Officinal extract of nux vomica should require about 14.8 cc. of 0.01 n-NaOH. So the alkaloids present have neutralized 35.2 cc. of 0.01 n-HCl, corresponding to 16 per cent.

3. Tincture of Nux Vomica.—Evaporate in a weighed dish, after adding 1 cc. of dilute sulphuric acid (1 + 4), 50 grams of tincture of nux vomica to 10 grams. Transfer the residue to an Erlenmeyer flask, rinsing the dish with 5 grams of absolute alcohol. Add 20 grams of chloroform, shake well, and then add 2 cc.

of sodium hydroxide solution and 5 cc. of sodium carbonate solution previously used in rinsing the dish. Allow the mixture to stand for one hour, shaking well from time to time. From this point the procedure is the same as that for extract of nux vomica. Continue from the place in the latter marked with an asterisk (*), beginning with the words "Then add 50 grams of ether, shake well . . . " Also at the place marked with two asterisks (**) read "to the combined chloroform extracts 40 cc. of 0.01 n-HCl . . . " instead of 50 cc. required in case of extract of nux vomica.

Calculations.—The German Pharmacopoeia assumes that nux vomica and its preparations contain equal parts of strychnine and brucine. Since both alkaloids are monacid bases, their equivalent and molecular weights are the same. So the mean equivalent weight of equal molecules of strychnine and brucine is

$$\frac{(C_{21}H_{22}O_2N_2 + C_{23}H_{26}O_4N_2)}{2} = \frac{334 + 394}{2} = \frac{728}{2} = 364$$

Hence 1000 cc. of 0.01 n-HCl neutralize 3.64 grams of strychnine-brucine and 1 cc. of 0.01 n-HCl neutralizes 0.00364 gram of alkaloidal mixture.

Example.—In the assay of tincture of nux vomica the alkaloidal mixture from 33.33 grams of tincture is dissolved in 40 cc. of 0.01 n-HCl. If excess of acid requires for neutralization 17.1 cc. of 0.01 n-NaOH, then $40 - 17.1 = 22.9$ cc. of 0.01 n-HCl are required to neutralize alkaloids present. This volume of 0.01 n-HCl neutralizes $22.9 \times 0.00364 = 0.08336$ gram of mixed alkaloids. So 100 grams of tincture contain $3 \times 0.08336 = 0.25$ gram of mixed strychnine-brucine.

ASSAYS OF CRUDE MATERIALS AND PREPARATIONS NOT IN THE GERMAN PHARMACOPOEIA

Estimation by Sulphate Method of Quinine in Cinchona Bark and Preparations

(Carles[1])

Because of simplicity and accuracy, this method is to be preferred to other procedures recommended for the same purpose. Therefore Hille[2] advises its use, especially for practical purposes. However, it fails to give accurate results when the mixture of cinchona alkaloids contains less than 20 per cent. of quinine. But this condition is not likely to arise in the case of true cinchona barks, or their extracts and tinctures, for according to Hille when bark contains 5 per cent. of total alkaloids, at least 3 per cent. is quinine. So at least 60 per cent. of total alkaloids in cinchona bark is quinine. In this case the sulphate method is applicable. Difference in solubility of sulphates of cinchona alkaloids in ammonium sulphate solutions forms the basis of separation. Solubility in water at 15° of the sulphate in question according to E. Schmidt is:

[1] P. Carles: Zeitschr. f. analyt. Chem., 9 (1870), 467.

[2] See the critical discussion by W. Hille as to the different methods of estimating quinine together with the other cinchona alkaloids. Archiv. d. Pharmazie 241 (1903), 54.

Quinine sulphate..... 1:800; Cinchonine sulphate..... 1:65
Quinidine sulphate..... 1:100; Cinchonidine sulphate..... 1:97

Guareschi found that quinine sulphate, practically speaking, is practically insoluble in ammonium sulphate solution. Hille has confirmed this result. A slight correction is necessary for the 20 cc. of water used in washing quinine sulphate, that is, an addition of 0.0078 gram to the weight of quinine sulphate obtained.

Procedure. 1. Cinchona Bark.—Put 12 grams of finely powdered cinchona bark dried at 100° in a separating funnel and add 90 grams of ether, 30 grams of chloroform, and 10 cc. of sodium hydroxide solution. Allow the mixture to stand for 3 hours, shaking well from time to time. Then add 10 cc. of water, or enough to cause cinchona powder when well shaken to gather into balls and leave the supernatant chloroform-ether solution perfectly clear. After 1 hour filter 100 grams of the latter through a well-covered, dry paper into a weighed dry flask. Evaporate the chloroform-ether solution and dry the flask at 110° to constant weight. The increase in weight of the flask is the weight of total alkaloids in 10 grams of bark. With gentle heat dissolve in the flask the mixed alkaloids thus obtained in 10–12 cc. of 10 per cent. sulphuric acid. Filter this solution through a small paper to remove any insoluble matter. Treat the residue once more with 2 cc. of 10 per cent. sulphuric acid, passing it through the same paper. Neutralize the combined filtrates with ammonia upon the water-bath until very faintly acid (the entire volume should be about 40 cc.). Shake well from time to time and allow to stand for 16 hours. Then collect precipitated quinine sulphate, $(C_{20}H_{24}O_2N_2)_2.H_2SO_4$, upon a weighed paper dried at 100°, or better in a dried and weighed Gooch crucible. Wash with 20 cc. of cold water and dry at 100° to constant weight. Make a correction of 0.0078 gram of quinine sulphate for the 20 cc. of wash-water.

Calculation.—The weight of quinine in 10 grams of cinchona bark is found by the following proportion:

$$\underset{(746)}{(C_{20}H_{24}O_2N_2)_2.H_2SO_4} : \underset{(648)}{(C_{20}H_{24}O_2N_2)_2} = \text{Weight of quinine sulphate} + 0.0078 : x$$

2. Cinchona Extract.—In a measuring cylinder dissolve 3 grams of aqueous cinchona extract in 5 cc. of water and 5 cc. of absolute alcohol. Add 50 cc. of ether, 10 cc. of chloroform, and after shaking well 10 cc. of sodium carbonate solution (1:3). Shake from time to time during 3 hours. When two layers have formed, add enough ether to bring the ether layer to the 75 cc. mark. Carefully rotate the container and evaporate 50 cc. of clear chloroform-ether layer in a weighed dry flask. Dry for 1 hour at 105° and weigh when cold. The increase in weight is the weight of total alkaloids in 2 grams of cinchona extract. It should be at least 0.12 gram = 6 per cent. of alkaloids.

To determine quinine, pour very dilute sulphuric acid over the weighed residue in the flask, warm and filter. Rinse the flask several times with very dilute sulphuric acid, dilute to about 40 cc. and proceed as directed above under cinchona bark (1). After 2 hours collect quinine sulphate upon a weighed paper, or in a weighed Gooch crucible. The calculation is the same as for cinchona bark.

Estimation of Colchicin in Colchicum Seed and Corms

(Katz and Bredemann[1])

Exhaust colchicum seed or corms with 60 per cent. alcohol and evaporate 50 grams of this extract to 20 grams. Add 0.5 gram of solid paraffine and 20 cc. of water. Warm until the paraffine is melted and alcohol has been completely expelled. Cool the liquid evaporated to 10–15 cc. and filter through moist paper. Melt the paraffine cake with 10 cc. of 10 per cent. acetic acid upon the water-bath and pass the cold liquid through the same paper. Wash the latter, the paraffine cake, and the dish with water. Saturate the total filtrate with sodium chloride and extract first with 20 cc. of chloroform and then with 10 cc. portions until a few drops of the aqueous liquid show scarcely any turbidity with 0.05 n-iodine solution. Pass the chloroform solution through paper moistened with this solvent and evaporate. To expel chloroform retained by colchicin, dissolve the residue in a little water and filter. Evaporate the solution in a weighed dish and dry the residue over sulphuric acid to constant weight.

Note.—Using this method, Bredemann obtained the following percentages of colchicin:

In seed	0.046–0.13	per cent.
In corms	0.032–0.06	per cent.
In fresh flowers	0.6	per cent.
In dry flowers	1.8	per cent.

Picrolonate Method of Estimating Hydrastine in Hydrastis Root and Extract

(Matthes and Rammstedt[2])

The German Pharmacopoeia requires estimation of hydrastine but not of berberine and phytosterol, physiologically inert substances also present in hydrastis preparations. The mixture of ether and petroleum benzine used by the German Pharmacopoeia dissolves hydrastine and phytosterol but not berberine. If the mixture is then shaken with dilute hydrochloric acid, hydrastine passes into the acid but phytosterol remains dissolved in the ether mixture.

Estimation of hydrastine by picrolonic acid appears simpler than by the method of the German Pharmacopoeia, because picrolonic acid does not precipitate phytosterol and consequently separation of the latter from hydrastine by hydrochloric acid is not necessary. Matthes and Rammstedt obtained nearly pure hydrastine picrolonate from hydrastis extract. Its melting-point was 220–225° as compared with 225° for hydrastine picrolonate, $C_{21}H_{21}NO_6 \cdot C_{10}H_8N_4O_5$, prepared from pure hydrastine.

1. Fluid Extract of Hydrastis.—Evaporate upon the water-bath in an Erlenmeyer flask 15 grams of fluid extract of hydrastis to about 5 grams. Add 10 cc of water, 10 grams of petroleum benzine, 50 grams of ether, and 5 cc. of ammonium hydroxide solution (10 per cent. NH_3). Shake well for 10 minutes. After the mixture has stood for 20 minutes, pour 40 grams of benzene-ether solution through a double creased paper and evaporate in a beaker to about half the volume. Then add 10 cc. of 0.1 n-picrolonic acid solution. After 24 hours

[1] Pharmazeut. Zentral-Halle 42,289 and Apotheker-Zeitung 18,817.

[2] See statements on page 585.

collect hydrastine picrolonate in a weighed Gooch crucible, wash with 2 cc. of alcohol-ether mixture (1 + 3), dry for 30 minutes at 105° and weigh.

2. Hydrastis Root.—Shake well for 30 minutes 6 grams of powdered root with 50 grams of ether, 10 grams of petroleum ether, and 6 cc. of ammonium hydroxide solution (10 per cent. NH_3). Then add 6 grams of water, shaking well until the upper layer of liquid is clear. Quickly filter 50 grams of ether-petroleum benzine solution and evaporate in a beaker to about half the volume. Then add 5 cc. of 0.1 n-picrolonic acid solution. After 24 hours filter the picrolonate precipitate and wash with only 1 cc. of alcohol-ether mixture (1 + 3). Otherwise the procedure is the same as described in 1.

In the calculation use the above formula of hydrastine picrolonate (Molecular Weight 647).

Estimation of Berberine.—To determine percentage of slightly physiologically active berberine in extract of hydrastis, add 20 grams of dilute sulphuric acid (1:5) to 10 grams of extract and allow the mixture to stand for 24 hours at as low a temperature as possible, preferably in an ice-box. Almost complete crystallization of berberine as difficultly soluble acid sulphate, $C_{20}H_{17}NO_4.H_2SO_4$, takes place. Collect the crystals upon a weighed Gooch crucible, washing first with a little water containing sulphuric acid and then with a little pure cold water. Dry at 100° to constant weight.

The crystalline deposit, frequently observed in extract of hydrastis, consists mostly of berberine mixed with a little phytosterol. This deposit is said to contain only traces of hydrastine (W. Meine).

Estimation of Caffeine in Coffee, Tea, Cola Nuts, and Guarana Paste

1. Keller's Method.[1]—Put 6 grams of dry, unbroken tea leaves[2] in a wide-mouth separating funnel and add 120 grams of chloroform. Shortly after add 6 cc. of ammonium hydroxide solution (10 per cent. NH_3) and shake well from time to time during 30 minutes. Then allow the separating funnel to stand at rest, until the solution is perfectly clear and the tea leaves have absorbed all the water. This may take 3–6 hours, or even longer, depending upon the variety of tea. Filter 100 grams of clear chloroform extract, representing 5 grams of tea, through a small paper moistened with chloroform. Collect the filtrate in a small weighed flask and distil the chloroform upon the water-bath. Pour 3–4 cc. of absolute alcohol over the residue, heating upon the water-bath to remove alcohol and expelling alcohol vapor with a hand bellows. In a few minutes caffeine will be dry and at the same time free from imprisoned chloroform. To some extent this treatment with alcohol also separates caffeine from extraneous chlorophyll. The latter adheres to the bottom and sides of the flask, whereas caffeine forms a white incrustation. Obtained in this manner caffeine is usually impure from

[1] C. C. Keller: Estimation of Caffeine in Tea. Berichte d. Deutsch. pharmaz. Ges. 7 (1897), 105.

[2] If a separating funnel with a wide neck is not available, the tea may be ground somewhat merely for the purpose of more easily rinsing from the funnel after extraction. Grinding tea too finely, however, not only is unnecessary but is even a disadvantage, for the yield of caffeine is not increased and the resulting solutions are too highly colored.

small quantities of ethereal oil, fat, vegetable wax, but principally from chlorophyll. For this reason it should be purified. Set the flask upon a boiling water-bath, pouring a mixture of 7 cc. of water and 3 cc. of alcohol over the crude caffeine. The latter almost instantaneously passes into solution upon agitation. Then add 20 cc. of water, stopper the flask, and shake well. Chlorophyll will gather in lumps and the solution will filter easily. Filter the caffeine solution through a small paper wet with water, washing flask and paper with 10 cc. of water. Evaporate the total filtrate to dryness upon the water-bath in a weighed glass dish and weigh the residue of nearly pure caffeine. The weight of this residue multiplied by 20 will give the percentage of caffeine in the tea.

Note.—Ammonia causes tea leaves to swell considerably, combining at the same time with tannic acid present. Caffeine is set free and dissolved by chloroform. The color of the chloroform extract depends upon the variety of tea. Fine, black teas (Pekoe, Souchong and Congo) give clear, pale green to yellowish green solutions. Teas not so black, or green teas, give dark and more brownish green solutions. In assaying those varieties of tea that probably contain only a small quantity of caffeine, take 12 grams and extract with 150 grams of chloroform. As the result of numerous experiments, Keller showed that the best and most expensive varieties of tea contain most caffeine. The average percentage of caffeine, based upon 50 assays of tea, was found to be 3.06. A green tea gave the lowest yield (1.78 per cent. of caffeine); and a Pekoe tea the highest yield (4.24 per cent. of caffeine).

Gadamer has found that Keller's method of estimating caffeine in tea may be applied also to coffee and cola preparations. Keller's method is especially useful in the case of roasted coffee. Though somewhat brown, the caffeine is always sufficiently pure.

2. Hilger-Juckenack Method.[1]—Macerate for several hours 20 grams of finely ground coffee, or tea, with 900 cc. of water in a large beaker at room temperatures. Then boil thoroughly, replacing water lost by evaporation. Raw coffee requires 3 hours and roasted coffee and tea 1.5 hours. Cool (60–80°), add 75 grams of aluminium acetate solution (see note, page 617), and then gradually, while stirring, 1.9 grams of sodium bicarbonate. Boil about 5 minutes and bring the total weight when cold to 1020 grams. Filter 750 grams (= 15 grams of original material), adding to the clear filtrate 10 grams of precipitated and powdered aluminium hydroxide and some filter paper made into a magma by agitation with water. Stir frequently and evaporate upon the water-bath. Thoroughly dry the residue in an air-closet at 100° and extract for 8–10 hours with pure tetrachloromethane (CCl_4), using a Soxhlet apparatus (Fig. 58). Tetrachloromethane, which is always colorless, is finally distilled and the residue of perfectly white caffeine dried at 100° and weighed. The results thus obtained are usually accepted without question. But if an absolutely accurate result is required, nitrogen in the crude caffeine may be determined by Kjeldahl's method. The quantity of anhydrous caffeine is calculated on the basis of this analysis. Commercial tetrachloromethane is usually impure and cannot be used directly

[1] A. Hilger and A. Juckenack: Estimation of Caffeine in Coffee and Tea. Forschungsberichte über Lebensmittel und ihre Beziehungen zur Hygiene 4 (1897), 49, 145.

in the extraction. It should be shaken 3–4 times with sodium carbonate solution, then several times with water, dried over fused calcium chloride, and distilled fractionally. Pure tetrachloromethane boils at 76–77°. The equivalent weight of caffeine = 0.25 $C_8H_{10}O_2N_4$ = 194:4 = 48.5. 1 cc. of 0.1 n-acid corresponds to 0.00485 gram of anhydrous caffeine.

3. Trillich-Goeckel Modification of Hilger's Method.[1]—Exhaust with water 10 grams of coffee ground as finely as possible. This will require 3 extractions with boiling water, using 200 cc. portions and heating each for 30 minutes. Combine the filtered extracts, cool and dilute to 495 cc. Add 5 cc. of basic lead acetate solution, shake well, filter 400 cc. and pass hydrogen sulphide through the filtrate. Dilute this filtrate to 500 cc., shake well and again filter 400 cc. This filtrate represents 6.4 grams of coffee. Concentrate this filtrate upon the water-bath, add 1 gram of magnesium oxide and sand, and evaporate to dryness. Triturate the residue and extract for 30 hours with acetic ether, using a Soxhlet apparatus. Evaporate the acetic ether extract in a Kjeldahl flask, or distil the solvent and determine nitrogen in the residue by Kjeldahl's method. 1 gram of nitrogen according to the proportion: 56 (4N): 194 ($C_8H_{10}O_2N_4$) = 1: x, corresponds to 3.4643 grams of caffeine. Crude caffeine is easily decomposed by the acid used in the Kjeldahl process in presence of mercuric oxide. Roasted coffee may easily give too much caffeine by this method, because the bases formed by roasting coffee, pyridine for example, are also extracted by acetic ether.

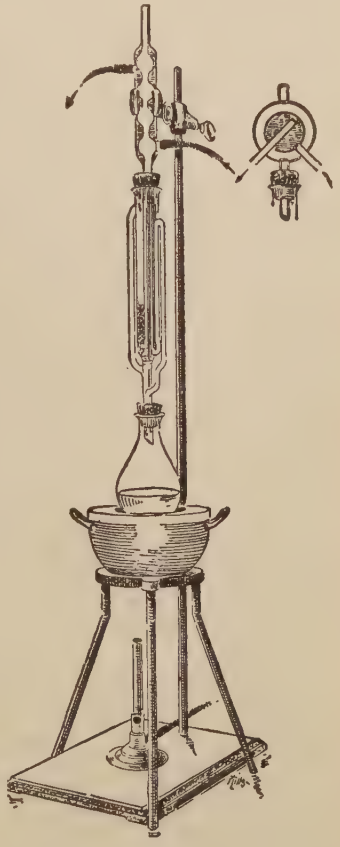


FIG. 58.—Soxhlet apparatus.

4. Trillich-Goeckel Modification of Socolof's Method.[1]—Put 10 grams of finely ground, undried coffee into a separating funnel provided with a plug of glass wool for a filter, and moisten with ammonium hydroxide solution. Let the mixture stand for 30 minutes and extract for 12 hours with 200 cc. of acetic ether, shaking frequently. Filter and wash three times with 50 cc. portions of acetic ether. Distil acetic ether upon the water-bath and boil the residue with milk of magnesia. Filter and evaporate the filtrate to dryness upon the water-bath. Dissolve

[1] H. Trillich and H. Goeckel: Contributions to the Knowledge of Coffee and Coffee Substitutes. Forschungsberichte über Lebensmittel und ihre Beziehungen zur Hygiene 4 (1897), 78.

residual caffeine in acetic ether or chloroform. Filter this solution into a weighed dish or Kjeldahl flask. Evaporate solvent and weigh caffeine, or calculate percentage from weight of nitrogen found. The latter method is more accurate. According to C. Wolff,[1] the residue from the acetic ether, or chloroform extract, should not be accepted as pure caffeine. Determination of nitrogen in this residue by Kjeldahl's method is the more reliable way of estimating caffeine in the extract.

5. Katz's Method.[2]—This method is based upon the fact that chloroform will extract caffeine quantitatively from a solution that is ammoniacal or faintly acid with hydrochloric acid.

Shake 10 grams of powdered coffee, or tea, for 30 minutes with 200 grams of chloroform and 5 grams of ammonium hydroxide solution. When the liquid has settled, filter 150 grams of the chloroform solution through a Sander's filter which will give a perfectly bright filtrate free from water. Distil chloroform completely and dissolve the residue with gentle heat in about 6 cc. of ether. Add 20 cc. of 0.5 per cent. hydrochloric acid and, in an assay of coffee, also 0.2–0.5 gram of solid paraffine. Evaporate ether and filter the cold aqueous solution. Wash flask and paper a few times with small portions of 0.5 per cent. hydrochloric acid. Finally extract total aqueous hydrochloric acid solution four times with 20 cc. portions of chloroform. Distil the filtered chloroform extract, dry the residue and weigh. This residue will consist of nearly pure caffeine. Katz found the following percentages of caffeine:

	Caffeine	Average
Raw coffee beans	0.9 –1.27 per cent.	1.14 per cent.
Dried cola nuts	1.51–1.94 per cent.	1.68 per cent.
Black tea	2.51–3.56 per cent.	3.07 per cent.
Guarana paste	2.83–4.74 per cent.	4.08 per cent.

Since the hydrochloric acid method described above fails to give entirely satisfactory results in estimating caffeine in Paraguay tea, or maté, Katz recommends the following procedure:

The tea triturated as finely as possible is treated with ammonium hydroxide solution and chloroform, as described above, and the residue from chloroform dissolved in ether. Add water to the ether and evaporate. Warm the aqueous solution for 10 minutes upon the water-bath with 2 cc. of lead hydroxide suspended in water (1:20). Should there be difficulty in getting a clean filtrate from this liquid, add a little calcined magnesium oxide. Usually this treatment gives a filtrate that is perfectly clear when cold and but slightly colored. Chloroform extracts quite pure caffeine from this solution. By this method maté yields 0.3–1.6 per cent. of caffeine, the average being 0.71 per cent.

6. Dieterich's Method of Estimating Total Alkaloids (Caffeine and Theobromine) in Cola Nuts.[3]—Moisten 10 grams of finely grated cola nuts with a

[1] C. Wolff: Zeitschr. f. öffentl. Chemie, 12, 186.

[2] E. Katz: Quantitative Estimation of Caffeine. Ber. d. Deutsch. Pharmaz. Ges. 12 (1902), 250.

[3] K. Dieterich: Assay of Cola Nuts and Cola Extract. Pharmaz. Zeitung 42 (1897), 647.

little water, mix with 10 grams of granulated, unslaked lime and extract with chloroform in a Soxhlet apparatus for 45 minutes. Evaporate the extract almost to dryness and dissolve the residue with gentle heat in 20 cc. of normal hydrochloric acid. Filter and dilute to 100 cc. Add to this filtrate ammonium hydroxide solution in large excess, shake frequently during 15 minutes, and extract three times with 20 cc. portions of chloroform. Evaporate this chloroform solution in a weighed flask and dry the residue, which consists usually of perfectly pure caffeine, at 100° to constant weight.

This method may also be used in estimating caffeine in tea, or in Paraguay tea. Mix the finely ground material with unslaked lime and extract with chloroform in a Soxhlet apparatus. In the case of tea the caffeine is pure, white and free from chlorophyll.

Estimation of Nicotine in Tobacco

1. Kissling's Method.[1]—First remove the ribs and then cut the tobacco leaves into small pieces. Dry 1–2 hours (50–60°) and then reduce to a uniform, coarse

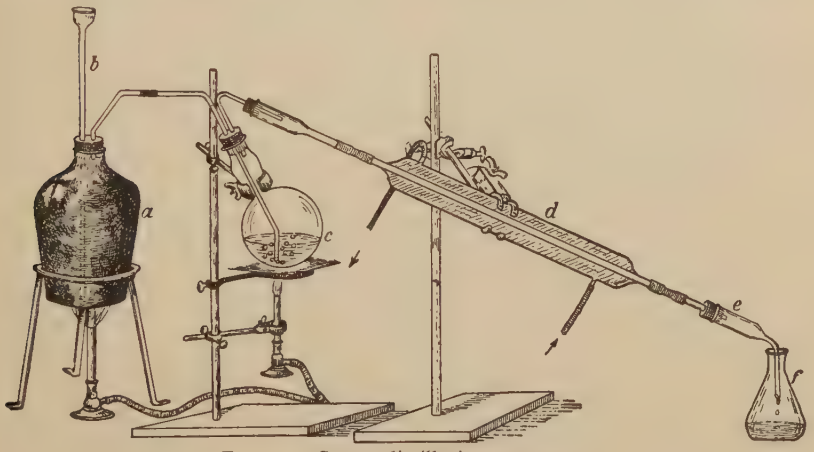


FIG. 59.—Steam distillation apparatus.

powder. Triturate 20 grams of this powder with 10 cc. of dilute, alcoholic sodium hydroxide solution (6 grams of sodium hydroxide dissolved in 40 cc. of water and 60 cc. of 95 per cent. alcohol). Transfer this moist powder to a paper thimble and extract 2–3 hours with ether in a Soxhlet apparatus. Carefully distil the ether solution so that a portion of solvent remains. Add 50 cc. of very dilute sodium hydroxide solution (4 grams of sodium hydroxide in 1000 cc. of water) to the residue and distil with steam, arranging the apparatus as shown in Fig. 59.

Begin introducing steam after the nicotine solution has been boiling several minutes. Collect about 400 cc. of distillate, that is, continue distilling until

[1] R. Kissling: Estimation of Nicotine and Ammonia in Tobacco. Zeitschrift f. analyt. Chemie 34 (1895), 731 and 21, 76.

the distillate is no longer alkaline. Mix well, add a few drops of rosolic acid solution to the distillate, and titrate nicotine with 0.1 n-sulphuric, or oxalic acid, until the red color has just disappeared.

Apparatus.—Steam is generated in a tin or copper can (a) provided with a long funnel-tube (b) extending nearly to the bottom. A 3–4 liter glass flask, containing a few pieces of pumice-stone to prevent bumping, may be used instead of the can. The material examined is put into a capacious round-bottom flask (c). The latter is connected with a long condenser (d) at the end of which is an adapter (e) leading into the receiver (f).

Calculation.—Although nicotine, $C_{10}H_{14}N_2$ (162), as a di-acid base can combine with two equivalents of acid, it behaves upon titration, with rosolic acid or iodeosine as indicator, as if it were a monacid base with the equivalent weight 162. 1000 cc. of 0.1 n-acid consequently correspond to 16.2 grams of nicotine.

2. Keller's Method.[1]—In this method tobacco is saturated with sodium hydroxide solution and nicotine set free is extracted with a mixture of ether and petroleum ether. Pour 60 grams of ether and 60 grams of petroleum ether over 6 grams of dry tobacco in a 200 cc. Erlenmeyer flask. Add 10 cc. of 20 per cent. aqueous sodium hydroxide solution and allow the mixture to stand half an hour, shaking well from time to time. After the mixture has stood at rest for 3–4 hours, pour 100 grams of ether solution through a small creased paper, receiving the filtrate in a 200 cc. Erlenmeyer flask. The solution contains nicotine together with a little ammonia which must be removed before titration. By means of a hand bellows and a glass tube extending to the bottom of the flask force a current of air through the solution so that there is considerable agitation. It requires about 1.5 minutes to expel all ammonia. At the same time 8–10 grams of ether evaporate. Add 10 cc. of alcohol, a drop of 1 per cent. iodeosine solution, and 10 cc. of water to the ammonia-free solution. Stopper the flask and shake well. Nicotine and iodeosine dissolve in the water which has a red color. Add a slight excess of 0.1 n-HCl, enough to discharge the color, and titrate excess of acid with 0.1 n-NaOH. The quantity of nicotine in tobacco shows a wide variation and ranges from 0.6 to 4.8 per cent.

3. Chapin's Silico-tungstic Acid Method.[2]—Silico-tungstic acid is a sensitive reagent for nicotine. Bertrand places the limit of delicacy at 1:300,000, and Chapin has found that the test is even more delicate, if conducted in a solution faintly acid with 0.1 per cent. hydrochloric acid. Chapin also determines nicotine quantitatively by precipitating with excess of aqueous 12 per cent. silico-tungstic acid solution in a solution rendered faintly acid with hydrochloric acid, filtering and washing the precipitate which is finally ignited or dried at 120° to constant weight. The product of ignition consists of silico-tungstic anhydride ($12WoO_3.SiO_2$) and the precipitate dried at 120° of anhydrous nicotine silico-tungstate having the composition ($2C_{10}H_{14}N_2.2H_2O.SiO_2.12WoO_3$).

Procedure.—The weight of material taken for examination should contain from 1–2 grams of nicotine. A convenient weight of tobacco extract is 10

[1] C. C. Keller: Estimation of Nicotine in Tobacco. Berichte d. Deutsch. pharm. Ges. 8 (1898), 145.

[2] R. M. Chapin: Estimation of Nicotine in Nicotine Solutions and Tobacco Extracts. U. S. Dept. of Agriculture, Bureau of Animal Industry. Bulletin 133 (1911), 1, 4.

grams. Put the weighed substance in a capacious, round-bottom flask, preferably a Kjeldahl flask, dilute to a thin liquid with about 20 cc. of water, add 10 cc. of 30 per cent. sodium hydroxide solution, also 1.5 grams of paraffine, and distil with steam (Fig. 59), using a Kjeldahl spherical glass still-head to connect distillation flask and condenser. During distillation the volume of liquid in the glass flask (c), which is heated with a low flame, should be kept as nearly constant as possible. A liter flask containing 50 cc. of 10 per cent. hydrochloric acid serves as receiver. The end of the adapter should dip under the surface of the acid and the condenser should be kept as cold as possible. For complete absorption of nicotine vapor, the current of steam should be quite gentle, especially at the beginning of distillation. When distillation is completed, the volume of liquid remaining in the flask (c) should be as small as possible. As soon as the volume of distillate is about 1000 cc., collect an additional 50 cc. in another receiver and test with silico-tungstic acid to make sure that no nicotine is distilling over. Thoroughly mix the 1000 cc. of distillate, measure out 100 cc., and precipitate nicotine with 10 cc. of 12 per cent. aqueous silico-tungstic acid. Stir for half an hour, collect the precipitate in a weighed Gooch crucible upon an asbestos mat, wash with 0.5 per cent. hydrochloric acid until the filtrate no longer turns blue upon addition of a little zinc dust (test for tungstic acid), ignite strongly for 10 minutes and weigh. The residue from ignition consists of $SiO_2.12MoO_3$. Its weight multiplied by 0.1139 gives the quantity of nicotine in 100 cc. of distillate. The final result is not affected by allowing the precipitate to stand longer, for nicotine is completely precipitated at once by silico-tungstic acid. Consequently the precipitate may be filtered off as soon as formed. Small quantities of ammonium salts, occurring in tobacco extracts, are without influence upon the composition of the precipitate.

4. Rasmussen's Method of Estimating Nicotine in Tobacco and Tobacco Extracts.[1]—Rasmussen critically examined the different methods of estimating nicotine and of the extraction methods gives the preference to that of Keller. He combines this method somewhat modified with the precipitation method of Bertrand-Chapin in which ammonia is without effect.

Procedure.—Thoroughly mix 10 grams of dry, powdered tobacco in a porcelain mortar with 8 cc. of a mixture of 3 parts of "soap lye" and 1 part of alcohol. By means of a glass spatula transfer the moist mixture completely to a wide-neck flask, adding 50 cc. of ether and 50 cc. of petroleum ether. Stopper the flask and allow the mixture to stand for 5 hours, shaking from time to time. Then filter the liquid through a dry, creased paper covered with a glass plate into a 100 cc. flask. When this is a little more than half full, transfer 50 cc. of filtrate with a pipette to a separating funnel and extract three times with 25 cc. portions of 1 per cent. hydrochloric acid. It is advisable to use a separating funnel with a short stem cut off at an angle that may be washed within and without by means of a spray from a wash-bottle. The extractions usually require in all about 5 minutes. Precipitate nicotine in the aqueous hydrochloric acid extract with 12 per cent. aqueous silico-tungstic acid, 10 cc. of this reagent usually being sufficient. A test, however, should always be made with a few drops of the reagent to make sure that all nicotine is precipitated. The precipitate is allowed to

[1] H. B. Rasmussen: Estimation of Nicotine in Tobacco and Tobacco Extracts. A Critical Investigation. Zeitschr. f. analyt. Chemie 55 (1916), 81.

stand for 10 hours and then collected either upon a dry Gooch crucible, weighed in a weighing-tube, or upon an ash-free paper. It should be washed with 1 per cent. hydrochloric acid until 10 cc. of filtrate fail to show any precipitate with a few drops of 1 per cent. nicotine solution. The precipitate in the Gooch crucible is dried at 120° to constant weight (about 1 hour) and the crucible weighed in the weighing-tube. The weight of precipitate, multiplied by $20 \times 0.1012 = 2.024$ (log. = 0.30615), gives the percentage of nicotine in the leaves. If the precipitate is collected upon an ash-free paper, both are dried in a weighed platinum crucible. All separated carbon is carefully burned over a Bunsen burner and the crucible is then strongly ignited 5–7 minutes (not longer) over a Teclu burner. The crucible is allowed to cool in desiccator for about half an hour and then weighed. The weight of ignited residue, multiplied by $20 \times 0.1140 = 2.28$ (log. = 0.35794), gives the percentage of nicotine in the tobacco.

In the examination of a tobacco extract, carefully weigh 3–4 grams to one mg., put in a 100 cc. flask, and then add 5 cc. of soap lye, 5 cc. of water, 25 cc. of ether and 25 cc. of petroleum ether. Shake well and allow to stand 4–5 hours. Then measure 25 cc. of ether-petroleum ether mixture and extract three times with 25 cc. portions of 1 per cent. hydrochloric acid. The further procedure is like that already described above. In the calculation it should be remembered that, when a grams of extract are weighed, nicotine in $a/2$ grams of extract is determined.

Notes.—The advantages of this method are the following: 1. By the ether mixture nicotine is quickly and completely extracted and from this solution is completely removed by 1 per cent. hydrochloric acid. 2. A loss of nicotine through evaporation of ether extract is excluded. 3. Ammonia, which is the critical-point in all determinations of nicotine by titration, has no place in this method of determination. 4. The analysis is quickly carried out and requires very little attention. Since anhydrous nicotine silico-tungstate is very hygroscopic, the crucible with precipitate should be weighed in a closed weighing-tube. Chapin recommends this method as very accurate, but also remarks that the ignition method, which is more quickly carried out, suffices for ordinary use. Precipitation of nicotine silico-tungstate, if made with stirring, is complete within half an hour and the precipitate thus obtained is finely crystalline.

If the tobacco extract contains pyridine, this must first be completely removed by steam distillation after addition of 100 cc. of water and 10 cc. of glacial acetic acid. The collection of 800 cc. of distillate should be sufficient to remove all pyridine. Since nicotine is a much stronger base than pyridine, only the latter will pass over under the conditions of distillation. When the contents of the flask are cold, add 15 cc. of 50 per cent. sodium hydroxide solution and distil nicotine as above.

Tobacco Smoke.—Nicotine is practically the only constituent of tobacco smoke that increases blood-pressure. The amount of nicotine in smoke, according to van Lecumen,[1] does not depend upon a cigar being mild or strong, nor upon its color, nor even upon the quantity of nicotine in the tobacco. The harm caused by the smoke of a given kind of cigar, so far as this runs parallel with increase of blood-pressure, can be established only by a direct determination of the amount

[1] W. S. van Lecumen: The Nicotine-content in Smoke of Heavy, Light and Nicotine-free Cigars. Archiv. f. experiment. Pathol. u. Pharmakol. 84 (1919), 282.

of nicotine in the smoke. Experiments upon decapitated cats, to determine increase of blood-pressure brought about by the products caught in acetic acid from cigar and cigarette smoke, show that smoke from mild cigars sometimes contains more bases producing increase of blood-pressure than that from strong cigars. Using a physiological method of determination, van Lecumen found the percentage of nicotine in the smoke from Wendt's patented cigars, said to be non-injurious to health, as high as in that from ordinary mediumly strong cigars.

Estimation of Pilocarpine in Jaborandum Leaves[1]

1. Fromme's[2] Method.—Extract 15 grams of rather finely powdered jaborandum leaves with 150 grams of chloroform and 15 grams of ammonium hydroxide solution (10 per cent. NH_3), shaking frequently for 30 minutes. Filter this mixture through a large, smooth paper, covering the funnel with a glass plate. As soon as the chloroform drops slowly, add a little water and filtration will become more rapid. After collecting a full 100 grams of filtrate, add about 1 gram of water, shake well and set aside. The water takes up fine particles of powder that have passed through the paper, leaving the chloroform solution quite clean. After 1 hour weigh 100 grams of chloroform solution (= alkaloids in 10 grams of jaborandum leaves).

Fromme directs extracting the 100 grams of chloroform solution successively with 30, 20 and 10 cc. of 1 per cent. hydrochloric acid which dissolves pilocarpine (and isopilocarpine) as hydrochlorides. Extract this acid solution first with 20 cc. of ether, to remove fat and resin. Then add excess of ammonia and extract free alkaloids successively with 30, 20 and 10 cc. of chloroform. Pass the combined chloroform extracts through a dry paper, evaporate in a weighed flask, dry the residue at 100° and weigh.

2. Matthes and Rammstedt's[3] Method.—Evaporate in a beaker to about 20 cc. the 100 grams of chloroform solution obtained as described above. Add first 3 cc. of 0.1 n-picrolonic acid and then 60 cc. of ether. After 24 hours collect the precipitate of pilocarpine picrolonate in a weighed Gooch crucible, wash with 1 cc. of alcohol-ether mixture (1 + 3), dry at 110° and weigh. Pilocarpine picrolonate thus obtained (= pilocarpine from 10 grams of jaborandum leaves), $C_{11}H_{16}N_2O_2.C_{10}H_8N_4O_5$ (Mol. Wt. 472) melts at 200–205°.

Piperine and Estimation in Pepper

Black pepper is the dried, unripe fruit of the pepper plant (Piper nigrum L.), whereas the ripe fruit deprived of its outer covering is the white pepper of commerce. The actual constituents of pepper are piperine, an ethereal oil (oil of pepper), and a resin called chavicine. In rather large doses pepper is toxic.[4]

[1] See the article upon pilocarpine, page 194.

[2] Caesar and Loretz: Geschäftsbericht, 1901, 27.

[3] See page 585.

[4] In his "Intoxikationen" R. Kobert cites the case of three young swine, each of which received a tablespoonful of powdered pepper. Severe intestinal inflammation ensued and two died. The toxic action of pepper is due to piperine, for the ethereal oil of pepper, according to R. Kobert, is not concerned with absorptive toxic action.

Preparation of Piperine.—Extract finely divided white pepper with 90 per cent. alcohol and distil the latter from the extracts. Treat the residue with cold sodium hydroxide solution which dissolves the resin but not piperine. Wash the residual piperine with water and crystallize from hot alcohol, using animal charcoal to remove color. White pepper contains 7–8 per cent. of piperine.

Piperine, $C_{17}H_{19}NO_3$, crystallizes in colorless, shining, rectangular, monoclinic prisms melting at 128–129°. When pure it is nearly tasteless but impure piperine has a sharp, burning taste. It is nearly insoluble in water, freely soluble in alcohol, and also soluble in ether, benzene and chloroform. Piperine is a very weak base, dissolving in dilute mineral acids with almost as much difficulty as in pure water. A solution of piperine in concentrated sulphuric acid has a ruby color, soon changing to dark brown and gradually to greenish brown and fading upon addition of water. Concentrated nitric acid converts piperine into an orange-red resin soluble with blood-red color in dilute sodium hydroxide solution. No salts of piperine with acids are known.

Prolonged heating with alcoholic potassium hydroxide solution decomposes piperine into the potassium salt of piperic acid and piperidine:

$$\begin{array}{l} CH = CH.CO.NC_5H_{10} \\ | \\ CH = CH.C_6H_3(O_2CH_2) \end{array} + KOH = C_5H_{10}NH + \begin{array}{l} CH = CH.COOK \\ | \\ CH = CH.C_6H_3(O_2CH_2) \end{array}$$

Piperine — Piperidine — Potassium piperate

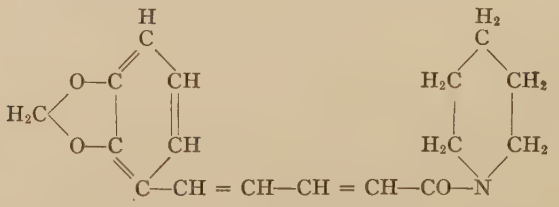
Rügheimer[1] synthesized piperine by putting together these two products. Piperic acid was first converted into its chloride by means of phosphorus pentachloride (α). Piperyl chloride was then condensed with piperidine (β) in benzene solution:

$$\alpha.\begin{array}{l} CH=CH.COOH \\ | \\ CH=CH.C_6H_3(O_2CH_2) \end{array} + PCl_5 = \begin{array}{l} CH=CH.COCl \\ | \\ CH=CH.C_6H_3(O_2CH_2) \end{array} + POCl_3 + HCl$$

$$\beta.\begin{array}{l} CH=CH.COCl \\ | \\ CH=CH.C_6H_3(O_2CH_2) \end{array} + HNC_5H_{10} = \begin{array}{l} CH=CH.CO.NC_5H_{10} \\ | \\ CH=CH.C_6H_3(O_2CH_2) \end{array} + HCl.$$

Piperyl chloride — Piperidine — Piperine

On the basis of the known structure of piperic acid and piperidine, piperine must have the following constitution:



[1] L. Rügheimer: Synthetic Piperine. Berichte d. Deutsch. chem. Ges. **15** (1882), 1390.

Estimation of Piperine in Pepper

1. Koenig's Method.—Exhaust 10–20 grams of pepper, ground as finely as possible, in a Soxhlet apparatus with strong ethyl or methyl alcohol, or petroleum ether. Distil the alcohol or petroleum ether. The residue consists of piperine and resin. Shake this residue with cold sodium carbonate solution to dissolve the resin. Filter from undissolved piperine and wash the latter with cold water. Dissolve in alcohol or petroleum ether, evaporate the filtered solution in a weighed flask or dish, and dry the residue at 100° to constant weight.

To determine resin in pepper at the same time, filter the sodium carbonate solution from crude piperine and add hydrochloric acid to the filtrate. Filter the precipitated resin, redissolve in alcohol in a weighed flask, evaporate solvent, and dry the residue in desiccator to constant weight.

2. Cazeneuve and Caillot's Method.—Add enough water to make a thin mixture of powdered pepper with twice its weight of slaked lime and stir well. Heat to boiling in a porcelain dish, dry thoroughly upon the water-bath, and then extract with ether in a Soxhlet apparatus. Distil ether in a weighed flask and dry the residue of piperine at 100° to constant weight. To obtain pure crystalline piperine, dissolve the residue from ether distillation in the least possible volume of boiling alcohol, surround the solution with ice, collect piperine upon a weighed filter, and dry at 100° to constant weight. This purification of piperine is attended with more or less loss and consequently the result is only approximately correct.

Estimation of Santonin in Wormseed[1]

1. Thaeter's[2] Method.—Extract 10 grams of crushed wormseed in a Soxhlet apparatus with ether for 12 hours. Distil ether and boil the residue for an hour with 5 grams of lime and about 300 cc. of water. Replace water lost by evaporation. Filter hot and wash the residue with water. Faintly acidify the filtrate with sulphuric acid and warm gently until santonin crystals begin to form. Then add 100 grams of aluminium acetate solution,[3] heat the mixture to boiling, and finally evaporate to dryness upon the water-bath. Mix the finely powdered residue with 3 grams of magnesium oxide, moisten this mixture with

[1] Wormseed (Flores cinae) consists of the unexpanded flower-heads of Artemisia cina which are 3–4 mm. in length.

[2] K. Thaeter: Qualitative and Quantitative Detection of Santonin in the Flower-heads of Artemisia Maritima. Archiv d. Pharmaz. 235 (1897), 401; 237 (1899), 626; 238 (1900), 383.

[3] Dissolve 300 parts of aluminium sulphate in 800 parts of water; add acetic acid (sp. gr. 1.041 = 30 per cent.) 360 parts; triturate calcium carbonate 130 parts with 200 parts of water, and add this mixture slowly with continued stirring to the first solution; set the whole aside for 24 hours without applying heat and stir occasionally; then strain, press the precipitate without washing and filter the liquid. It is a clear, colorless liquid, having the sp. gr. 1.044–1.046, a faint odor of acetic acid, an acid reaction, and a sweetish, astringent taste. National Dispensatory.

a little water, and again bring quickly to dryness. Powder the mixture as finely as possible, dry at 105°, and extract in a Soxhlet apparatus with anhydrous, acid-free ether for 5 hours. Santonin is deposited upon distilling ether as a faintly yellowish residue which is then dried at 100° to constant weight.

Notes.—When wormseed is heated with lime, santonin passes into solution as calcium santonate and at the same time resinous substances are saponified. Dilute sulphuric acid liberates first santonic acid which passes at once into its inner anhydride, santonin. Basic aluminium acetate, produced by boiling, precipitates resinous and colored substances. Finally magnesium oxide serves to neutralize free acetic acid. Under the conditions, practically no magnesium santonate is formed. Thaeter obtained 88–92 per cent. of the santonin present. Wormseed contains about 2.5 per cent. of santonin.

2. Katz's[1] Method.—According to Katz, there are two sources of error in Thaeter's method: 1. Even after one hour's boiling of santonin with aqueous milk of lime, complete formation of salt does not take place. 2. Addition of magnesia reconverts a large part of santonin set free into magnesium santonate. So Katz recommends the following procedure: Extract 10 grams of coarsely powdered wormseed in a Soxhlet apparatus with ether for 2 hours. Distil the ether. A dark green resin weighing 1.5–2 grams usually remains. Boil this residue 15–30 minutes under a reflux with 5 grams of crystallized barium hydroxide dissolved in 100 cc. of water. Cool and without filtering render the solution acid to litmus with carbon dioxide. Filter immediately with suction to remove barium carbonate, and wash the precipitate twice with 20 cc. portions of water. Evaporate the pale yellow solution to about 20 cc. in a dish upon the water-bath. Add 10 cc. of 12.5 per cent. hydrochloric acid, continue heating upon the water-bath exactly 2 minutes longer, and pour the solution into a separating funnel. Dissolve santonin crystals left in the dish in about 20 cc. of chloroform. Pour this solution into the separating funnel and shake well. When the solutions have separated clear, pass the chloroform through a dry filter. Wash dish, separating funnel and paper 2–3 times with 10 cc. portions of chloroform. Distil the chloroform and boil the residue 10 minutes under a reflux with 50 cc. of 15 per cent. alcohol. Filter while hot into a weighed flask, and wash flask and paper twice with 15 cc. portions of 15 per cent. boiling alcohol. Cover the flask and set aside in the cold for 24 hours. Weigh flask and contents and pass the latter through a weighed paper, disregarding the milky appearance of the filtrate caused by minute resinous drops. Wash flask and paper once with 10 cc. of 15 per cent. alcohol, dry the paper in the flask and weigh both. Finally apply a correction on account of the solubility of santonin in the alcohol used. Each 10 grams of filtrate contain 6 mg. of santonin. Santonin by this method is crystalline and usually faintly yellow. Katz found the quantity of santonin in wormseed to vary between 1.21 and 3.16 (average 2.42) per cent. This method is based upon the fact that the santonin in 10 grams of wormseed is easily soluble in 50 cc. of hot 15 per cent. alcohol, whereas only very little resin is dissolved. As this dilute alcoholic solution cools, santonin crystallizes almost quantitatively.

[1] J. Katz: Quantitative Estimation of Santonin. Archiv d. Pharmaz. 237 (1899), 245.

Santonin Troches.—To estimate the quantity of santonin in troches, made from this substance and sugar, extract the finely ground mixture direct with hot chloroform. Santonin usually may be weighed without further purification.

Chocolate Santonin Troches.—In a somewhat simpler form the method described above may be used to estimate santonin in chocolate troches. Weigh 3–4 troches and boil 15 minutes under a reflux with 5 grams of barium hydroxide and 100 cc. of water. Saturate the liquid when cold with carbon dioxide. Filter, wash the residue with water, and evaporate the brownish filtrate to 100 cc. Warm the liquid and add 10 cc. of dilute hydrochloric acid. Three extractions with chloroform yield nearly pure santonin. To get santonin crystals almost white and ready for weighing, evaporate the chloroform solution and expel the last traces of chloroform by adding a few cc. of ether. If santonin is impure from traces of fatty acids, boil once with 10 cc. of petroleum ether and filter when cold. Santonin is nearly insoluble in cold petroleum ether.

Santonin may be detected and estimated in toxicological analysis in a similar manner. Acidify the material with hydrochloric acid, extract with chloroform, and treat the chloroform residue with barium hydroxide solution as described above.

Chemical Estimation of Strophanthin in Strophanthus Seeds and Tincture

(Fromme[1])

(For Further Information about Strophanthin See Pages 431 to 435)

The chemical methods in the literature of estimating the value of Strophanthus seed and its tincture require first removal of fatty oil, tannin and coloring matter. Strophanthin is then estimated gravimetrically or polarimetrically; or it is isolated and converted by hydrolysis with dilute acid into strophanthidin and from the weight of the latter the percentage of strophanthin is deduced. Fromme employed the second method.

Lampart and Müller,[2] after comparing and critically examining the various methods proposed of estimating strophanthin in Strophanthus seed and tincture, showed experimentally that Fromme's method of 1910 (1911) gives the highest values. The conclusion is that this method makes possible a more complete exhaustion of the drug. These authors consider it the most complete and reliable method available at the present time. A possible disadvantage is its length. On the other hand, the reliability of its results, which agree well with those obtained by physiological means, is a decided advantage. Reference is also made to the colorimetric estimation of K-strophanthin given on page 433.

1. Estimation in Strophanthus Seed

Procedure.—Put into an Erlenmeyer flask 7 grams of finely crushed strophanthus seed with 70 grams of absolute alcohol. Get the gross weight and heat to boiling for 1 hour in a steam-bath under a reflux. Cool, bring to the original gross weight with absolute alcohol, and filter. Expel alcohol from 50.5 grams of

[1] G. Fromme: Geschäftsbericht of Caesar and Loretz, 1905, 99 and 1910, 118.

[2] L. B. Lampart and A. Müller: Estimation of Strophanthin in Strophanthus Seed and Tincture of Strophanthus. Archiv d. Pharmazie, 251 (1914), 609.

filtrate = 5 grams of seed in a 10 cm. porcelain evaporating dish upon a steam-bath. Pour petroleum ether over the residue and pass this through a 5 cm. smooth, dry paper, washing both dish and paper with some petroleum ether. Removal of the last traces of fatty oil in the seed is unnecessary. Petroleum ether should remove only the main portion. Wash the insoluble matter upon the paper into the dish with about 5–8 cc. of boiling water. Heat the contents of the dish to boiling, add 5 drops of basic lead acetate solution, and also about 0.2 gram of infusorial earth. Mix well and pass 100 cc. of the contents through a 5 cm. paper into an Erlenmeyer flask. Wash both dish and paper with small quantities of boiling water until finally the filtrate does not taste bitter.

To hydrolyze strophanthin, add 5 drops of hydrochloric acid to the filtrate. Keep at a gentle boil for 2 hours upon an asbestos plate over a small flame. After the volume has been reduced to about 10 cc. by evaporation, bring to 20 cc. with distilled water. When cold, extract successively with ten 10 cc. portions of chloroform and filter the individual chloroform extracts into a 100 cc. tared Erlenmeyer flask. As above, again heat the water solution separated from chloroform to boiling for half an hour. Cool and repeat the extraction with ten 10 cc. portions of chloroform. If the aqueous liquid, after expelling chloroform by heat, still tastes bitter, again heat to boiling for half an hour and repeat extraction with chloroform as before. Combine the filtered chloroform extracts in a small weighed flask and distil the chloroform. Dry the residual K-strophanthidin with the flask in vacuum desiccator (see page 96) to constant weight. Since 1 part of strophanthidin, according to Fromme, corresponds to 2.187 parts of pure strophanthin, the weight of the flask residue must be multiplied by this factor to get the weight of strophanthin in 5 grams of strophanthus seed.

Notes.—If the flask with strophanthidin is dried in vacuum desiccator, adhering chloroform is completely removed in about 3 hours, especially if the vacuum is renewed from time to time. In that case there is no need of drying flask and contents upon a water-bath at about 80°. When pure, K-strophanthidin crystallizes from methyl alcohol in shining, monoclinic prisms melting at 169–170°. It dissolves readily in alcohol, fairly easily in chloroform, with difficulty in ether, and is almost insoluble in water. Concentrated sulphuric acid dissolves K-strophanthidin producing a brick-red solution.

2. Estimation in Tincture of Strophanthus

Heat 51 grams of tincture (= 5 grams of seed) in a porcelain dish upon the water-bath to expel alcohol. Dissolve the residue in about 20 cc. of hot water. Add 15 drops of basic lead acetate solution, 0.2 gram of infusorial earth, and proceed exactly as described above in the estimation of strophanthin in strophanthus seed.

The Bodinus[1] Modification of Fromme's Method

1. Tincture of Strophanthus.—Evaporate 25 cc. of tincture in a platinum dish upon the water-bath. Dissolve the residue in 15 cc. of hot water, add 3 drops of

[1] Bodinus: Estimation of Strophanthin in Tincture and Seed. Apotheker-Zeitung 34 (1910), 72.

basic lead acetate solution, and collect the precipitate upon a paper, washing with hot water. To hydrolyze strophanthin, heat the 50–60 cc. of filtrate, after adding 10 drops of 25 per cent. hydrochloric acid, to boiling for 2 hours under a reflux. Evaporate the turbid liquid in a platinum dish upon the water-bath with considerable sand and some animal charcoal and extract the fine, dry mass with hot chloroform upon an asbestos filter connected with suction-pump. Distil the chloroform in a weighed flask and dry residual strophanthidin at 100° to constant weight, or better in vacuum desiccator (see Fig. 13 on page 96). One part of strophanthidin corresponds to 2.187 parts of strophanthin.

2. **Strophanthus Seed.**—Extract fat from 1 part of coarsely powdered strophanthus seed in a Soxhlet apparatus with petroleum ether. Then extract for 1 hour under a reflux with 10 parts of 70 per cent. methyl alcohol. Bring the extract to a definite volume with 70 per cent. methyl alcohol and treat an aliquot part of the solution as described above for tincture of strophanthus. Take 3–5 grams of strophanthus seed for the determination.

Estimation of Alkaloids in Nux Vomica

(Keller[1])

Remove fat from nux vomica by treating 15 grams of well-dried and finely powdered drug in a 250 cc. Erlenmeyer flask two or three times with 30 cc. portions of ether. Shake well for 5 minutes. Pour these ether extracts into a flask and, since they contain a little alkaloid, extract dissolved alkaloid with 5 cc. of 0.1 n-hydrochloric acid and 10 cc. of water. Repeat the extraction of the ether layer, separated from the aqueous solution, using water instead of acid. Add 100 cc. of ether, 50 grams of chloroform, and 10 grams of 10 per cent. ammonium hydroxide to powdered nux vomica free from fat. Shake well for 30 minutes and add to this mixture the hydrochloric acid solution used in extracting alkaloid from the first ether washings. Again shake well and, when the liquids have separated clear, pour 100 grams of ether-chloroform solution through a small paper into a weighed Erlenmeyer flask. Distil the chloroform and ether as completely as possible. Usually the alkaloids appear as colorless varnishes that persistently retain chloroform. To remove the latter, pour a few cc. of absolute alcohol upon the residue and expel completely upon the water-bath. Repeat this treatment 2–3 times. This will give crystalline alkaloids that may be dried at 100° to constant weight.

Estimation of Alkaloids in Nux Vomica and Preparations by Picrolonic Acid[2]

(Matthes and Rammstedt)

Nux vomica contains strychnine and brucine upon the average in equal quantity combined with tannic acid. Alkaline hydroxide or carbonate solutions liberate the alkaloids from their salts. The free bases are then extracted with ether-chloroform mixture. The solvent is reduced to smaller volume by evaporation or distillation and alkaloids are precipitated with picrolonic acid.

[1] Celebration of the fiftieth anniversary of the foundation of the Swiss Druggists Association. Abstract in Zeitschr. f. analyt. Chem. 23 (1894), 491.

[2] See statements on pages 585 to 587.

Strychnine picrolonate, $C_{21}H_{22}N_2O_2.C_{10}H_8N_4O_5$ (Mol. Wt. 598) melts with decomposition at 286°.

Brucine picrolonate, $C_{23}H_{26}N_2O_4.C_{10}H_8N_4O_5$ (Mol. Wt. 658) melts with decomposition at 277°.

1. **Extract of Nux Vomica.**—Dissolve 1 gram of extract in 5 grams of absolute alcohol and 5 cc. of water. Shake well with 50 grams of ether and 20 grams of chloroform. Add 10 cc. of sodium carbonate solution (1 + 2) and again shake well for 10 minutes. Let the mixture stand at rest for 20 minutes. Pass 50 grams of chloroform-ether mixture through a dry, double, creased paper and evaporate half of the solvent in a beaker. Add about 5 cc. of 0.1 n-alcoholic picrolonic acid to the warm solution. A yellow crystalline precipitate of strychnine and brucine picrolonates soon appears. After 24 hours collect the mixed picrolonates upon a weighed Gooch crucible. Wash excess of picrolonic acid from the precipitate with 2 cc. of an alcohol-ether mixture (1 + 3), dry 30 minutes at 110°, cool in desiccator and weigh.

Calculation.—Use the mean molecular weight of brucine and strychnine picrolonates (628) and also the mean molecular weight of brucine and strychnine (364). The proportion is:

Grams picrolonate mixture	:	Grams strychnine and brucine	=	Wt. of precipitate	: x
628		364			

Since the quotient 364:628 = 0.5798, the weight of mixed alkaloids is obtained by multiplying the weight of picrolonate precipitate by this quantity. This precipitate represents total alkaloids in 0.666 gram of extract of nux vomica, for only two-thirds (50 grams) of the original 75 grams (5 grams of alcohol + 50 grams of ether + 20 grams of chloroform) of solvent, containing alkaloids in 1 gram of extract of nux vomica, were used.

2. **Tincture of Nux Vomica.**—Evaporate 50 grams of tincture in an Erlenmeyer flask to 10 cc. Cool and shake well with 5 cc. of absolute alcohol, 50 grams of ether and 20 grams of chloroform. Add 10 cc. of sodium carbonate solution (1 + 2) and shake again for 10 minutes. After 20 minutes pass 50 grams (two-thirds of the original mixture) of ether-chloroform mixture through a double, creased paper. Evaporate half of the solvent in a beaker and add 5 cc. of 0.1 n-alcoholic picrolonic acid to the warm residue. Treat the picrolonate precipitate as described above.

3. **Nux Vomica.**—Exhaust 15 grams of powdered nux vomica, previously dried at 100°, by shaking well with 100 grams of ether and 50 grams of chloroform. Then add 10 cc. of a mixture of 2 parts of 15 per cent. sodium hydroxide solution and 1 part of water and shake again for 10 minutes. Add an additional 15 cc. of water, or enough to cause the powder to gather into balls after vigorous agitation and leave the supernatant ether-chloroform mixture clear. After 30 minutes pass the clear ether-chloroform solution through a dry, double, creased paper. Evaporate 50 cc. of filtrate in a beaker nearly to dryness and add a second 50 cc. portion of filtrate to the residue, bringing everything into solution (alkaloids from 10 grams of nux vomica). Add 5 cc. of 0.1 n-alcoholic picrolonic acid and treat the precipitate as previously described.

Estimation of Alkaloids in Nux Vomica and Preparations

(Azadian[1])

Alkalimetric estimation of nux vomica alkaloids, according to Azadian, gives very inaccurate results, whereas those obtained by using silico-tungstic acid are very reliable.

Procedure.—Extract for 2–3 hours 10 grams of powdered nux vomica in a Seiler extraction apparatus with a mixture of 50 grams of chloroform, 50 grams of ether and 5 grams of ammonium hydroxide solution, shaking frequently. Pour off the solution and make 2–3 more extractions. Combine the extracts, concentrate to smaller volume upon the water-bath, add 10 per cent. nitric acid, and shake in a separating funnel with a mixture of 10 cc. of dilute nitric acid and 20 cc. of water. Draw off the acid aqueous solution, shake the ether-chloroform solution with acid-free water, and expel upon the water-bath ether-chloroform mixture dissolved in the combined aqueous extracts. Cool and add 10 cc. of 5 per cent. silico-tungstic acid solution and 10 cc. of 10 per cent. nitric acid. Bring just to a boil, allow to stand in the cold for 24 hours, and collect the precipitate of brucine and strychnine silico-tungstate upon a 7 cm. ash-free paper. Wash with acid-free water, dry, ash, and ignite to constant weight. The weight of ignited residue of ($12\ WoO_3.SiO_2$) is multiplied by 0.498. This gives grams of alkaloids in the original material. This method is applicable to all nux vomica preparations (Azadian).

Quantitative Estimation of Strychnine in Presence of Brucine

(Gordin's[2] Modification of Keller's Method)

Strong nitric acid, gently heated with a solution of strychnine and brucine in 3 per cent. sulphuric acid, is without action upon the former alkaloid. But brucine is converted into non-basic substances not extracted by chloroform from alkaline solution.

Procedure.—Dissolve 0.2–0.3 gram of mixed alkaloids upon the water-bath in 15 cc. of 3 per cent. sulphuric acid and add 3 cc. of diluted nitric acid (equal parts of 68–69 per cent. acid (sp. gr. 1.42) and water) to the cold solution. Pour the mixture into a separating funnel after exactly 10 minutes and add sodium hydroxide solution in excess. Extract strychnine 3–4 times with chloroform. Pass the chloroform solution through a double filter into a small weighed flask, wash the filter with a little chloroform, add 2 cc. of pure amyl alcohol, and distil to dryness upon the water-bath. Remove the last traces of liquid, consisting mainly of amyl alcohol, by forcing a current of air through the flask warmed upon the water-bath. Finally dry the residue 2 hours at 135–140° and weigh when cold. In this way very pure white strychnine free from brucine is obtained.

Notes.—Ammonia, according to Gordin, can not be substituted for sodium hydroxide solution, as it gives colored strychnine. Amyl alcohol is added to the

[1] A. Azadian: Estimation of Alkaloids. Use in Case of Nux Vomica Preparations. Schweiz. Wochenschr. f. Chem. u. Pharmaz. 51 (1913), 761.

[2] H. M. Gordin: Quantitative Estimation of Strychnine in Mixtures of Strychnine and Brucine. Archiv d. Pharmaz. 240 (1902), 641.

chloroform solution to prevent strychnine crystals from being carried into the condenser by decrepitation during distillation.

Estimation of Theobromine and Caffeine in Cacao and Chocolate

(Prochnow's Modification of the Beckurts-Fromme[1] Method)

The chloroform extraction method is preferable to all other procedures, especially if use is made of the Katz extraction which permits of more rapid work. Addition to chloroform of phenol as a good solvent for theobromine is also to be recommended. The great advantage of the Fromme-Beckurts method is that it gives fat-free purine bases. Prochnow recommends the following procedure: Boil 6 grams of powdered cacao, or 12 grams of powdered chocolate, with 200 cc. of water and 3 cc. of dilute sulphuric acid in a tared liter flask for 30 minutes under a reflux. Then add 8 grams of calcined magnesia triturated with 400 cc. of water. Again boil for 1 hour under a reflux. Add 30–50 cc. of water, shake well, and determine the weight of flask contents, making allowance for the cacao or chocolate used. Allow to settle well and filter five-sixths of the determined weight of liquid (= 5 grams of cacao or 10 grams of chocolate) through a creased paper. Evaporate the filtrate upon the water-bath in a porcelain dish to about 10 cc. Add 2 grams of phenol to the residue and transfer to a Pregl or Gadamer extractor (see page 467), rinsing the dish with very little warm water. Then subject the mixture for at least 6 hours to extraction with chloroform heated in the weighed flask upon a vigorously boiling water-bath. Connect the extractor with a second flask containing chloroform, extract for 2 hours longer, and make sure by distilling the chloroform that no more theobromine remains to be extracted. Transfer to the flask as completely as possible chloroform remaining in the extractor. Finally distil the entire chloroform solution from the weighed flask. To remove phenol from the residue, continue heating upon the water-bath and carefully blow in air from a small bellows, or suck out the vapor by means of a pump, drying at 100° to constant weight. Analyses made in this manner gave the following percentages of theobromine: roasted cacao shells 0.578–1.380; unroasted cacao kernels 1.460–1.812; and roasted cacao kernels 1.536–1.880.

Further information about the chemistry of theobromine will be found on pages 412–415.

Separation of Theobromine from Caffeine

Cacao and preparations contain very little caffeine which is usually determined with theobromine. These two purine bases may be separated by means of carbon tetrachloride, for at room temperature theobromine is not dissolved appreciably, whereas caffeine is soluble.

Procedure.—Allow the weighed residue from chloroform to stand for 1 hour with 100 grams of carbon tetrachloride at room temperature. Shake occasionally and then filter. Distil carbon tetrachloride and extract the residue

[1] H. Beckurts: Cacao and Chocolate. Arch. d. Pharmaz., 244 (1906), 486; and A. Prochnow: Estimation of Xanthine Bases in Cacao and Chocolate. Arch. d. Pharmaz., 247 (1910), 698.

repeatedly with water. Evaporate the aqueous solution in a weighed dish and dry the residue (caffeine) at 100° to constant weight.

Repeatedly extract theobromine, insoluble in carbon tetrachloride, and also the paper with water. Filter, evaporate the total filtrate and weigh the residue (theobromine) dried at 100°.

Notes.—In the method described above, Beckurts and Fromme eliminate injurious effects due to concentration by boiling with dilute sulphuric acid. Xanthine bases are set free from combination with organic acid and recombined with sulphuric acid. Magnesia sets these bases free from their sulphates and at the same time holds back coloring matter and fat, thus eliminating these impurities.

Biological Estimation of Digitalis Leaves and Preparations[1]

Unquestionably a purely chemical method of adequately determining the value of digitalis leaves and preparations has not as yet been found. Obvious sources of error in the physiological tests must also be admitted, for these experiments make use of living animals that differ with regard to their life energy. Fränkel[2] in fact remarks that "the frog's heart tells us all that we can hope to learn from a pharmacological test-object, as far as estimating the value of digitalis preparations is concerned."

Of the different digitalis glucosides digitoxin is most potent in action. According to Gottlieb,[3] it occurs in digitalis leaves in soluble form, that is, as a glucotannoid, combined with tannic acid. Of the many proposed methods of estimating digitalis, that of Focke,[4] which Führer has thoroughly tested, may be briefly considered. A definite quantity of an infusion of digitalis leaves is injected into a green frog whose heart is exposed. The time required for arrest of the heart's action is then observed. Two equal portions of 10 per cent. infusion of digitalis, totalling a fortieth of the body-weight, injected into the lymph-sac of the upper part of the thigh of a green frog (weighing 20–30 grams) arrest the action of the heart in 9–11 minutes, as averaged from at least four experiments. According to Focke, the so-called "Valor" is derived from the equation: $V = \frac{p}{d.t}$ in which p is the weight of the frog, d the dose injected, and t the time that elapses between injection and arrest of the heart's action. For example, a frog weighing 24 grams receives 0.6 cc. of a 10 per cent. infusion and arrest of heart's action takes place in 10 minutes. Then $V = \frac{24}{0.6 \cdot 10} = 4.0$. This figure

[1] This article is included in this book for the sake of completeness. Such examinations cannot be conducted in a competent manner by chemists but only by pharmacologists. Precise instructions as to biological methods of estimation are given by H. Führer in his excellent book entitled "Nachweis und Bestimmung der Gifte auf biologischem Wege," 1911. (Biological Detection and Estimation of Poisons.)

[2] Fränkel: Therapy of the Present, March, 1902.

[3] R. Gottlieb and R. Tambach: Digipuratum. Münch. med. Wochenschr., 58 (1911), 10.

[4] C. Focke: Physiological Evaluation of Digitalis Leaves. Archiv d. Pharmaz., 241 (1903), 128 and 669; 245 (1907), 646; 247 (1910), 544; 248 (1910), 345.

is taken as the normal value. The technique of the test on the frog is completely described by H. Fühner (loc. cit.).

Infusion of Leaves Prepared According to Focke.—Dry powdered leaves for 30 minutes at 60° or 80° at the highest. Put 2 grams in a small porcelain ointment jar placed in a larger dish and pour into the latter enough boiling water to come up half-way on the jar. Then boil in a wide test-tube a scant 24 cc. of water, adding 8 drops of 5 per cent. sodium carbonate solution. Pour this weak alkaline solution boiling hot upon the powder. Stir quickly with a glass rod until all lumps are broken up. Cover tightly and allow extraction to proceed for 20 minutes. Filter through a piece of old handkerchief into a graduated medicine glass, squeezing the cloth as tightly as possible. Bring the volume of filtrate to the 20 cc. mark with water, thus obtaining a 10 per cent. infusion.

The filtrate is always turbid but that is immaterial. But if it should be too turbid, pass the infusion brought to a volume of 20 cc. again through another piece of cloth. Filtration through paper is not permissible, for active substances may be retained. Thus prepared, the infusion should be kept from sunlight and examined in a few hours at latest.

Remove alcohol from tincture of digitalis and from other alcoholic digitalis preparations by carefully concentrating upon a moderately warm water-bath and dissolving the residue in water. On the other hand, it is unnecessary to expel alcohol from tincture of strophanthus, for dilution with water, the degree of which according to Focke is quite important, serves the same purpose. To get the valor 100 with normal tincture of strophanthus (Kombé), the average strength of this tincture, dilute 1 cc. with 19 cc. of water.

In the procedure, according to Focke, the time elapsing between injection and arrest of the heart's action is the main factor in determining the value of digitalis preparations. On the other hand, Gottlieb[1] determines the smallest quantity necessary to produce arrest of the heart's action within 30 or at most 45 minutes, using in most cases green frogs of the same size weighing about 30 grams. The smallest dose may be taken as the unit and the potency expressed in terms of the number of such units per gram of preparation. A freshly prepared infusion of active digitalis powder must contain therefore at least 40–50 units to 1 gram of leaves. But freshly prepared infusion from 1 gram of the most potent leaves sometimes represents 120 units.

Constituents of Rhizome of Aspidium Filix-mas and Extract of Male Fern and Estimation of Filicic Acid in Ether Extract of Male Fern

The rhizome of Aspidium Filix-mas and its ether extract (Oleoresin of Male Fern) contain together with fatty oil several nitrogen-free and in part crystalline substances, only two of which appear to be anthelmintic in action. Boehm[2] in particular by his investigation of the rhizome of Aspidium Filix-mas and its

[1] R. Gottlieb: Physiological Evaluation of Drugs. Münchener med. Wochenschrift, 1908, No. 24.

[2] R. Boehm: Butyryl-Filicine. Ann. der Chem., 318 (1901), 230; Aspidinol. Ibid., 318, 245; Constitution of Flavaspidic Acid, Filicic Acid, Albaspidine, and Two Reactions of the Phloroglucinol Series. Ibid., 318,253; Also in addition Arch. f. experim. Pathol. u. Pharmakol. 38 (1897), 35.

extract has contributed largely from a chemical and pharmacological point of view to our knowledge of this drug. Up to the present time not less than seven different specific substances have been isolated. According to Kraft,[1] good extracts of male fern contain on the average: filicic acid 3.5 per cent.; flavaspidic acid 2.5 per cent.; albaspidin 0.05 per cent.; aspidinol 0.1 per cent.; flavaspidinin 0.01 per cent.; filmarone 5 per cent.; filixnigrins 6 per cent. Crystalline aspidin, according to Hausmann,[2] is found in extract of male fern only when the rhizome of Aspidium spinulosum is mixed with Aspidium filix-mas. Filico-tannic acid, always present in the rhizome of Felix-mas, is similar to quino-tannic acid. Ferric chloride solution imparts an olive-green color to its solutions. Hydrolysis with dilute acids decomposes it into glucose and filix red. In addition to the substances already mentioned, the rhizome of Filix-mas contains 0.04 per cent. of a light yellow ethereal oil having a fern-like odor.

Physiological Action.—The active substances in the rhizome of Felix-mas exert a toxic action upon both nerve and muscular tissue. Straub[3] showed that they are energetic protoplasmic poisons acting particularly upon the muscular tissue of worms and molluscs. The smooth muscular tissue of invertebrates is very sensitive to filicic acid. So the rhizome of male fern and its tincture probably act by producing paralysis of the muscular tissue of tapeworm. Filicic acid, on the other hand, in mammals acts as an irritant of the central nervous system, giving rise to muscular contraction and even to tetanic convulsions followed by muscular and heart paralysis and collapse. Severe poisoning has been repeatedly observed in man after large doses of extract of male fern, or in consequence of injudicious use of the drug following a fast. According to Meyer-Gottlieb, symptoms of gastro-intestinal irritation appear, such as nausea, vomiting and diarrhoea. In severer cases the stools may be bloody. Dizziness, fainting and convulsions have also been observed. Cardiac depression, irregularity in breathing, and temporary or permanent blindness may appear. Icterus has usually been observed when poisoning is of long duration. The observation of Straub that the poison is destroyed in the organism of higher animals may explain at least in part the relative non-toxicity of filicic acid to the body of the host.

The active constituents of the fern species are compounds of butyric acid and phenols of the phloroglucinol series. Other drugs that are specifics for tapeworm (anthelmintics) also contain phloroglucinol derivatives, as kosotoxin of Cusso and rottlerin of Kamala.

Filicic Acid or Filicin.—This substance gradually separates as a crystalline deposit when ether extract of Filix-mas is allowed to stand. It may be obtained pure by washing the granular precipitate with ether-alcohol and recrystallizing from hot acetic ether or methyl alochol. Various widely different formulae have been proposed for filicic acid. Boehm has assigned the empirical formula

[1] F. Kraft: Filmarone, The Constituent in Extract of Male Fern possessing Anthelmintic Properties. Arch. d. Pharmaz., 242 (1904), 489.

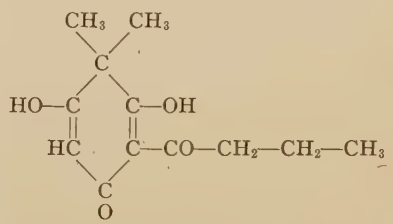
[2] A. Hausmann: Ether Extract of Male Fern. Arch. d. Pharmaz., 237 (1899), 544.

[3] W. Straub: Physiological Studies of the Substances of the Filicic Acid Group. Arch. f. exper. Pathol. u. Pharmak., 48 (1902), 1.

$C_{35}H_{38}O_{12}$. Pure filicin appears in small, pale yellow, rhombic plates melting at 184–185°. Repeated crystallization from hot acetic ether offers the best method of purification. Dry filicin crystals are nearly insoluble in water, methyl and ethyl alcohol, and ether, but very freely soluble in chloroform. Sometimes rather large rhombic plates separate, if acetone is added to a saturated chloroform solution of filicin. Filicic acid, heated for several hours with zinc dust and sodium hydroxide solution (15 per cent.), gives rise to such cleavage-products as normal butyric acid, phloroglucinol, $C_6H_3(OH)_3$, methyl, dimethyl and trimethyl-phloroglucinol, $C_6H_2(CH_3)(OH)_3$, $C_6H(CH_3)_2(OH)_3$, and $C_6(CH_3)_3(OH)_3$, as well as filicinic acid, $C_8H_{10}O_3$. Butyryl-filicinic acid is obtained as an intermediate product of the action of zinc dust and sodium hydroxide solution. It crystallizes in rhombic plates and is broken down by prolonged action of the same reagents into normal butyric acid and filicinic acid:

$$\underset{\text{Butyryl-filicinic acid}}{C_8H_9O_2(OC_4H_7O)} + H_2O = \underset{\text{Filicinic acid}}{C_8H_{10}O_3} + \underset{\text{Butyric acid}}{C_4H_8O_2}$$

Boehm has proposed three formulae for butyryl-filicinic acid, or filicinic acid-n-butanone, differing in the positions of the OH and CO groups. The following is one of these formulae:



Pharmacological interest in filicinic acid-n-butanone centres in the fact that it appears to be the specifically active principle among the substances of the filicic acid group. Though in less degree, it acts upon the animal organism in the same manner characteristic of the mother-substances, filicic acid, flavaspidic acid, albaspidin, and aspidin. This action ceases, however, with removal of the butyryl-radical, for filicinic acid is inactive. Poulson found crystalized filicic acid physiologically inactive. But if the latter is dissolved in sodium hydroxide solution and poured into an excess of hydrochloric acid, the physiologically active form, that is, amorphous filicic acid, possessing anthelmintic properties, separates out.

Flavaspidic Acid ($C_{24}H_{28}O_8$).—Extract of male fern contains 2 per cent. of this substance. In the purification of filicic acid it is found in the mother-liquor. Crystallized from methyl alcohol, it appears in long yellow plates or prisms. It exists in two forms having different melting-points. By fusion, or recrystallization from benzene or glacial acetic acid, one form may be converted into the other. The α-modification melts at 92° and in so doing passes into β-flavaspidic acid melting at 150°. The β-acid is more closely related to filicic acid and, when heated in alkaline solution with zinc dust, forms the same bodies obtained from filicic acid. Unlike filicic acid, however, flavaspidic acid never gives rise to phloroglucinol.

Albaspidin ($C_{25}H_{37}O_8$).—Extracts of male fern contain only very small quantities of this substance (about 0.05 per cent.). The rhizome of Aspidium spinulosum contains somewhat more. By recrystallization from glacial acetic acid it is obtained in fine colorless needles melting at 148–149°. Rather easily soluble in ether, it dissolves only in hot alcohol, acetone, glacial acetic acid, or petroleum ether, and even then with difficulty. Caustic alkalies dissolve it easily but alkaline carbonates only very slightly. Its alcoholic solution turns litmus only faintly red. Ferric chloride colors the same solution dark red.

Filmarone ($C_{47}H_{52}O_{16}$).—This is the constituent of extract of male fern that, according to Kraft, possesses anthelmintic properties. It is a yellow amorphous powder melting at about 60°. Insoluble in water, it dissolves with difficulty in alcohol and petroleum ether, but very readily in ether, chloroform, acetic ether and acetone. Added to its alcoholic solution, ferric chloride produces a red-brown precipitate. Dissolved in acetone, filmarone gradually undergoes decomposition into filicic acid and filix-nigrins which are amorphous, black-brown substances that are physiologically inert. Warmed with sodium hydroxide solution and zinc dust, filmarone yields normal butyric acid, butyryl-filicic acid, phloroglucinol and the methyl ether of methyl-phloroglucinol.

Estimation of Filicic Acid in Ether Extract of Filix-mas

(Fromme)

Shake 5 grams of well-mixed extract of male fern continuously for 5 minutes in a separating funnel with 30 grams of ether and 100 grams of 2 per cent. barium hydroxide solution. Allow the mixture to stand at rest. As soon as it has settled, weigh into an Erlenmeyer flask 86 grams of the clear lower aqueous solution = 4 grams of extract of male fern. Add 2 grams of 25 per cent. hydrochloric acid and extract successively with 25, 15, 10 and 10 cc. portions of ether. Filter the clear ether extracts through a small paper into a weighed flask and distil the ether. Dry the residue first at 40°, then at 80°, and weigh. The weight represents the quantity of crude filicic acid in 4 grams of extract of male fern. Good extracts of male fern should give about 25 per cent. of crude filicic acid by this method.

Estimation of Crude Cresol and Content in Cresolic Soap Solution (Lysol)[1]

1. Estimation of Meta-cresol in Crude Cresol

The German Pharmacopoeia prescribes that m-cresol be determined by Raschig's[2] method, the basis of which is the difference in behavior of the three cresols when nitrated. Meta-cresol alone gives a trinitro-derivative, $C_6H(NO_2)_3(CH_3)(OH)$, almost insoluble in cold water, whereas ortho- and para-cresol nitrated at boiling temperature in presence of excess of nitric acid are completely oxidized to oxalic acid. Small quantities of phenol present are nitrated to picric acid soluble in water. Success with this method depends upon having the cresol mixture completely sulphonated before nitration, so that the mixed sulphonic

[1] For additional information see pages 52–53.

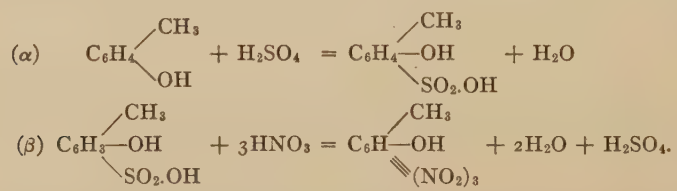
[2] F. Raschig: A Method of Estimating Meta-cresol in Cresol-mixtures. Zeitschr. f. angewandte Chemie, 1900, 759.

acids contain not a trace of free cresol; also upon bringing the sulphonic acids to the boiling temperature of the mixture (110–115°) as quickly as possible and in presence of excess of nitric acid. To this end the cresol-sulphuric acid mixture should be heated at 95–100° for at least 1 hour. The yield of trinitro-derivative from 1 gram of m-cresol is always 1.74 grams. The most varied mixtures of m-cresol with o- and with p-cresol, or with both, always gave the same results. Even when 9 grams of m-cresol were mixed with 1 gram of phenol Raschig obtained exactly $9 \times 1.74 = 15.6$ grams of trinitro-m-cresol. Only when the content of phenol exceeded 10 per cent. did picric acid separate with trinitro-m-cresol causing increase of weight. So the method fails when cresol mixtures are very rich in phenol. Otherwise results did not vary over 1 per cent. above or below the figure given above (Raschig).

Upon the average, commercial crude cresol contains 40 per cent. of ortho-, 35 per cent. of meta-, and 25 per cent. of para-cresol. Ortho-cresol boiling at 188° may be completely separated from crude cresol by very careful fractional distillation. The residual mixture then contains about 60 per cent. of meta- and 40 per cent. of para-cresol. These two cresols boil at nearly the same temperature (200°), so that their separation by fractional distillation is not possible. Though less toxic than phenol, the cresols are more powerful antiseptics. Of the three isomeric cresols, meta-cresol is said to be the least toxic but to have the strongest bacteriocidal action.

Estimation of Composition.—Heat 10 grams of crude cresol and 30 grams of concentrated sulphuric acid in a 1000 cc. wide-neck flask upon the water-bath for 1 hour. Bring the mixture to room temperature, add 90 cc. of commercial nitric acid (sp. gr. 1.380–1.400 = 58–60 per cent.), and at once shake carefully to dissolve. When the violent reaction that takes place after about 1 minute has subsided, allow the flask to stand for 15 minutes. Then pour the contents into a porcelain dish containing 40 cc. of water, and rinse the flask with the same volume of water. Two hours later break up with a pestle crystals that have formed. Filter the crystals by suction and wash with 100 cc. of water previously used to rinse the flask and dish, adding in small portions at a time. Dry crystals and paper for 2 hours at 100° and weigh when cold. It is convenient to use a paper of the same size as a counterpoise. In an analysis of officinal crude cresol the weight of trinitro-m-cresol thus obtained should be at least 8.7 grams. Its melting-point should not be below 105°. The quantity of m-cresol in officinal crude cresol amounts to at least 50 per cent.

Notes.—Heated with sulphuric acid, m-cresol is converted into m-cresol-sulphonic acid (α) which excess of nitric acid converts into trinitro-m-cresol (β):



Since poisonous nitrous fumes (NO_2) are given off abundantly in this reaction the operation should be conducted in a hood provided with a good draught.

2. Estimation of Total Cresol in Cresolic Soap Solution (Lysol)

Cresolic soap solution is a solution of crude cresol in potash soap made from linseed oil. It is a clear, red-brown, oil-like liquid that turns litmus paper blue, has the odor of cresol, and dissolves clear in water, glycerol, alcohol, and petroleum benzine. The quantity of crude cresol in cresolic soap solution must be approximately 50 per cent.

Estimation of Cresol.—Dilute 20 grams of cresolic soap solution with 60 cc. of water in a capacious flask, add dimethyl-amino-azo-benzene solution, and acidify with sulphuric acid to a red color. Then distil with steam.[1] At first the distillate is milky but as soon as it becomes clear turn off the water used for cooling. Continue distilling until steam begins to appear at the end of the condenser. Then begin cooling again and distil 5 minutes longer. Add 20 grams of salt to the distillate and shake well with 80 grams of ether. Separate the ether extract and distil in a weighed flask. Dry the residual cresol in the flask at 140° for 40 minutes and weigh. The weight of cresol obtained from officinal Liquor cresoli saponatus should be at least 9.5 grams. Cresol isolated from cresolic soap solution should meet the requirements established for crude cresol.

SUPPLEMENT

Detection of Blood-stains and Human Blood

1. Detection of Blood Stains

Detection of blood in dry stains upon fabrics, wood, knives, weapons, etc.,[2] is more certain and less open to question, if haemin crystals (Teichmann's blood crystals) are prepared from the blood-pigment. If haemin crystals are obtained, the stain in question may be regarded with certainty as due to blood. Fresh blood when dry is bright red and has a smooth surface. Flakes of such blood scraped from any material are garnet-red by transmitted light. A solution of fresh blood-stains in potassium or sodium hydroxide is dichroic, being red by transmitted and green by reflected light. Later dried blood becomes brownish red or dark brown. These color changes are due to conversion of oxyhaemoglobin into methaemoglobin and then into haematin. The first two substances are soluble in water but the last is not. Haematin, however, is soluble in alkalies and in alcohol containing sulphuric acid. This change of the blood-pigment depends not only upon the age of the stain but really upon the action of air (oxygen), light, heat, and moisture upon the blood before it is dry. If the blood is in a thin layer, haemoglobin will sometimes change into methaemoglobin even in 3–10 days. Boiling water causes immediate insolubility. The action is also very rapid in direct sunlight. Washing in alkaline solutions (boiling solutions of potassium or sodium soap, sodium carbonate solution, ammonia and sewage) also causes rapid decomposition. But acids, nitric and hydrochloric, as well as

[1] The apparatus (Fig. 59) on page 611 may be used for the distillation.

[2] The fact that blood mixed with iron oxide as, for example, blood upon rusty knives and weapons usually fails to give haemin crystals, should not be overlooked.

putrefaction, act more slowly, giving the blood a laked appearance and even making it clear and colorless. If, however, the blood has once dried, these injurious agencies, even putrefaction, act less easily.

Preparation of Haemin Crystals.—Prepare a cold aqueous extract of the stain as free as possible from fibers and evaporate the solution upon a watch-glass away from dust. Add a trace of sodium chloride[1] to the residue, also 8–10 drops of glacial acetic acid, and stir with a glass rod. Heat for an instant over a small flame, then evaporate the solution gradually upon a moderately warm water-bath and examine the residue with a microscope magnifying 300–500 times.

FIG. 60.—Haemin crystals.

If haemin crystals fail to appear, repeat the evaporation several times, using in each instance 8–10 drops of glacial acetic acid, and examine the residue each time under the microscope. Haemin crystals are brownish red to dark brown and form rhombic scales which frequently lie crossed (Fig. 60). Usually glacial acetic acid is the only solvent that will extract the pigment from old blood-stains. Brücke heats the stains or scrapings to boiling in a test-tube with 10–20 drops of glacial acetic acid. The decanted or filtered solution, after addition of a trace of sodium chloride, is evaporated upon a watch-glass to dryness at 40–80° and the residue is examined under the microscope. By this method it is immaterial whether blood has coagulated or not.

Cold water is without effect upon blood-stains, if they have previously been treated with hot water. Protein substances in blood are thus coagulated and rendered insoluble. In such a case treat the stain with water containing a few drops of sodium hydroxide solution. If the stains are upon wool, use very dilute sodium hydroxide solution since alkalies dissolve wool. Water containing ammonium hydroxide will extract stains and this alkali does not act upon wool. Use the alkaline aqueous extract to prepare haemin crystals. Evaporate the solution to dryness in a watch-glass upon the water-bath and mix the residue intimately with 8–10 drops of glacial acetic acid. Add a trace of sodium chloride and again evaporate. Sometimes it is advisable, after acidifying the extract of the stain with acetic acid, to add tannic acid, or zinc acetate, and prepare Teichmann's crystals from the precipitate.

Occasionally it is necessary to extract suspected stains with hot alcohol containing sulphuric acid. Haematin formed from blood-pigment dissolves.

[1] Strzyzowski (Chemisches Zentralblatt, 1897, I, 295) advises using sodium iodide instead of sodium chloride. Place a small particle of material suspected of containing blood upon a glass slide and add a drop of sodium iodide solution (1:500). Evaporate and cover with a cover-glass. Heat for 3–6 seconds with concentrated acetic acid allowed to run under the cover-glass. The test with this modification is said to be more delicate, owing to the darker color of the haematin hydriodide crystals. The crystals are usually obtained in less time and with as small a quantity as 0.000025 gram of fresh blood. Tr.

If this compound is present, the solution has a brown color. Excess of sodium hydroxide solution will produce dichroism characteristic of an alkaline haematin solution, namely, red by transmitted and green by reflected light. Obviously, haematin should be identified by the spectroscope both in acid and alkaline solution.

Blood mixed with iron oxide (blood upon rusty weapons) usually fails to give haemin crystals but the extract with dilute sodium hydroxide solution frequently shows the dichroism of haematin solution. Since iron oxide, or rust, forms an insoluble compound with haematin, warm such stains for some time upon the water-bath with sodium hydroxide solution to dissolve any haematin present.

Haematin.—Warming an aqueous blood solution to about 70° decomposes the blood-pigment oxyhaemoglobin into a protein substance called globin and haematin, a pigment containing iron. Acids, alkalies and several metallic salts decompose oxyhaemoglobin in the same way. If this decomposition takes place in the absence of oxygen, another pigment appears. Hoppe-Seyler gave the latter the name haemochromogen and other experimenters have called it "reduced haematin." Oxygen and consequently air rapidly oxidizes this pigment to haematin. On the other hand, reducing agents like ammonium sulphide convert haematin into haemochromogen. Different formulae for haematin are given. Kuster and others now give it the formula $C_{34}H_{34}N_4FeO_5$. Haematin is amorphous and has a dark brown or blue-black color. In water, dilute acids, alcohol, ether and chloroform it is insoluble but soluble in alcohol or ether containing acid. Even in very dilute solutions of caustic alkalies it is freely soluble. Alkaline haematin solutions are dichroic. In rather thick layers the color appears red by transmitted light and greenish in thin layers. Acid solutions are always brown. Alkaline haematin solutions are precipitated by calcium or barium hydroxide solution.

Haemin.—This substance is the hydrochloric ester of haematin. Very probably haemin has the empirical formula $C_{34}H_{33}N_4FeO_4Cl$.

Note.—If the blood-stain is perfectly fresh, it may be recognized by observing blood-corpuscles with the microscope. Human blood may be differentiated from animal blood by comparing blood-corpuscles with those of animal blood as to size, only when the corpuscles are still intact.

Spectroscopic Detection of Blood.—If the extract of a blood-stain with cold water is already brown, a third fainter and narrower band will appear in addition to the two oxyhaemoglobin bands. This lies in the orange between C and D and is the methaemoglobin band. Cold water will dissolve most of the methaemoglobin from fresh dried blood-stains.

Acetic acid will discharge these two bands, if the oxyhaemoglobin solution is not too dilute. At the same time the solution will become mahogany-brown from formation of haematin in acid solution. This solution has a characteristic spectrum, namely, four absorption-bands in the yellow and green. If excess of ammonium hydroxide is added to this solution, the alkaline solution contains haematin, recognizable by a broad, faint absorption-band lying between the red and yellow. A few drops of ammonium sulphide solution will extinguish this band and bring out two broad bands, namely, one in the green and the other in the light blue. These bands lie farther to the right than those of oxyhaemoglobin and are of about the same width. This is the spectrum of reduced

haematin (haemochromogen). All these spectroscopic tests are very characteristic, especially the spectra of oxyhaemoglobin, haemoglobin and, in the case of old blood-stains, that of reduced haematin. All these absorption-spectra are shown in Fig. 27 on page 328.

Up to the present time no red solution has been found that, upon abstraction and addition of oxygen, will give the same spectroscopic phenomena that blood gives.

When the quantity of blood is very small, or when the blood-pigment has undergone further decomposition, so that the bands of oxyhaemoglobin are no longer visible, it is advisable to extract the stains for several hours with concentrated potassium cyanide solution. Blood will give a light red or yellowish brown solution containing the cyano-compound of haematin. The spectrum of haematin in alkaline solution will appear as a broad, faint band.

The investigations of Kratter and Hammerl have shown that charred blood, that no longer responds to any of the other blood reactions, will still give the haematoporphyrin spectrum upon treatment with concentrated sulphuric acid (E. v. Hofmann, Lehrbuch der gerichtlichen Medizin, 1903).

Ammoniacal carmine solution gives two absorption-bands similar to those of oxyhaemoglobin but they do not change upon addition of acetic acid or ammonium sulphide. A band given by fuchsine analogous to that of haemoglobin remains unchanged after agitation with air.

Other Blood Tests

1. Schönbein-Van Deen Ozone Test.—A mixture of ozonized turpentine[1] and alcoholic tincture of guaiac resin, shaken with a little blood, produce a light blue color. Separated from turpentine, the tincture is deep blue. Though very delicate, this test is not characteristic of blood, for many inorganic and organic substances under the same conditions produce "guaiac blue." Nitrous acid, chlorine, bromine and iodine, chromic and permanganic acids, ferric and cupric salts produce blue solutions direct with guaiac resin. Usually in an examination of blood-stains it is possible to exclude these substances beforehand. But other substances such as cell-contents and haemoglobin, having the power of transferring ozone, may attach false significance to the guaiac-blue reaction. Enzymes (diastases), hydrolytic ferments (enzymes in the narrower sense), as well as the so-called oxidation ferments (oxidases), are organic substances of this character. They occur in different parts of plants, especially in fungi and seeds. Saliva, extracts of certain organs, contents of white blood-corpuscles, and pus cells are animal products of similar nature. Schaer[2] states that these animal and vegetable substances differ from hydrogen dioxide in being catalytic in action and carriers of oxygen at the same time. And also that a temperature of 100°, or contact with hydrocyanic acid, completely destroys their power of transferring oxygen, or at least greatly diminishes it, in which respect they are essentially

[1] Oil of turpentine always contains ozone when it has been exposed for some time to light in bottles that are not tightly stoppered.

[2] E. Schaer: Recent Observations with Regard to Detection of Blood by Means of the Guaiac Test. Forschungsber. über Nahrungsmittel, etc., 3 (1896), 1 and Archiv der Pharm. 236 (1898), 571.

different from haemoglobin. Neither high temperature (100°) nor hydrocyanic acid has any restraining influence upon haemoglobin as far as transference of oxygen is concerned. Consequently an extract, containing one of these ferment-like substances but no blood, placed even for a short time in a hot water-bath, loses the power of giving the "guaiac blue" test. In absence of blood, the result will also be negative, if the extract of the suspected stain is treated with hydrocyanic acid. For these reasons great care is necessary in interpreting a positive guaiac test given by the extract of a supposed stain. The guaiac test is certainly very useful as a delicate preliminary test and in many instances as a check upon blood. The three forms of the blood-pigment entering into such an examination, namely, haemoglobin, methaemoglobin and haematin, are alike in the guaiac test, at least qualitatively, as far as transference of oxygen is concerned. The examination and extraction of the stain may, therefore, be conducted in neutral, acid or alkaline solution, depending upon the nature of the substance, and either hot or cold. Render an alkaline extract faintly acid with acetic acid before adding guaiac tincture. In many instances it is advisable to extract the blood-stain with hot alcohol containing sulphuric acid. Treat such an acid, alcoholic haematin solution with guaiac tincture direct. Addition of water will precipitate the resin with the adherent blood-pigment.

(a) **Vitali's Procedure.**—Extract the stain with water containing carbon dioxide, or old stains with very dilute sodium hydroxide[1] solution free from nitrite and nitrate. Filter the extract and add a little alcoholic guaiac tincture to a portion of the filtrate after acidification with acetic acid, if necessary. If the milky liquid is not blue in 15 minutes, interfering oxidizing agents are absent. Then add a few drops of old turpentine and shake. The milky liquid will turn blue at once, or in a short time, if blood-pigment is present. Very gentle warming upon the water-bath increases the delicacy of the reaction. Even putrid blood 2 months old is said to give a positive test.

(b) **Schaer's Procedure.**—Blood-stains upon linen, though quite old, dissolve completely when treated for some time with 70 per cent. chloral hydrate solution. Moistening the stains beforehand with glacial acetic acid aids solution. Also prepare an extract of guaiac resin in 70 per cent. chloral hydrate solution. Mix the extract of the stain with an equal volume of the latter solution. In absence of nitrites, the color of this mixture is brownish yellow to light brown. If preferred, a contact test for blood may be made by this method. Add to the mixture of blood and guaiac Hünefeld's[2] turpentine solution, or hydrogen peroxide, as a surface-layer. An intense blue zone will appear where the two solutions meet. Guaiaconic acid in guaiac resin produces "guaiac blue." Döbner has suggested substituting a dilute solution of guaiaconic acid for guaiac resin. Blood, or blood-pigment, behaves like a ferment and activates the ozonized turpentine, or hydrogen peroxide, either of which by itself will not turn the solution of guaiac resin blue.

2. Schaer's Aloin Test.—The same conditions, producing "guaiac blue" from guaiaconic acid, give rise to "aloin red" from aloin. This substance has a stronger coloring power and lasts longer than "guaiac blue." Use the same

[1] Use for this purpose sodium hydroxide prepared from metallic sodium.

[2] See "Preparation of Reagents," page 642.

solution of blood in 70–75 per cent. chloral hydrate solution mixed with a weak chloral hydrate solution of aloin. Add Hünefeld's hydrogen peroxide solution as a surface-layer. After some time a violet-red zone will appear and a red color of equal intensity will gradually extend throughout the aloin solution. Another method of making this test consists in first extracting the blood-stain with pure water, acetic acid, chloral hydrate solution, or alkaline salt solution. Neutralize this solution and add dilute alcoholic aloin solution and hydrogen peroxide. If the suspected stain contains blood-pigment, a red color will appear at once and persist for a long time. The aloin test is inferior to the guaiac test in delicacy (Utz).

3. O. and R. Adler's[1] Blood Tests.—Leuco-malachite green = tetramethyl-diamino-triphenyl-methane, $C_6H_5\text{-}CH(C_6H_4N(CH_3)_2)_2$, and benzidine, $H_2N\text{-}C_6H_4\text{-}C_6H_4\text{-}NH_2$, are two very delicate reagents for blood-pigment. They may be used to detect blood-stains as well as blood in water, urine and faeces.

Thoroughly saturate the given stain with the reagent[2] and then pour upon it 3 per cent. hydrogen peroxide solution. If the stain is due to blood, it will at once assume an intense green color. Even blood-stains that have been boiled will give this test. In presence of iron salts the green color may not appear.

Prepare the reagent for the benzidine test by making a hot saturated alcoholic solution of pure benzidine, cooling and filtering. It is used as described below to detect blood in urine and faeces:

(a) **In Urine.**—Add 7.5 cc. of glacial acetic acid to about 15 cc. of urine. Haemoglobin, if present, is converted into haematin. Then extract with a few cc. of ether. A few drops of alcohol will break up the emulsion that forms. Separate the ether extract, adding a few cc. of benzidine solution, some 3 per cent. hydrogen peroxide solution, and a few drops of acetic acid. If the urine contains blood, a brilliant green color will appear. An alternative procedure is to add the leuco-malachite green reagent to the ether extract and some hydrogen peroxide. Any of the leuco-base that may be precipitated may be redissolved by means of glacial acetic acid.

(b) **In Faeces.**—Suspend faeces in some water and add to about 3 cc. of the unfiltered suspensate 2 cc. of benzidine solution, 2 cc. of 3 per cent. hydrogen peroxide solution and a few drops of acetic acid. If blood is present, an intense green color will appear.

[1] O. Adler and R. Adler: Behavior of Certain Organic Compounds toward Blood with Special Reference to Detection of Blood. Zeitschr. f. physiol. Chemie, 41 (1904), 59.

[2] The leuco-malachite green reagent is obtained by preparing a concentrated glacial acetic acid solution of the entirely colorless, chemically pure leuco-base of malachite green. To remove minute traces of green color even when an entirely colorless preparation is used, add to the solution the same volume of chloroform. Drop water into this mixture with careful shaking until chloroform is completely precipitated. Separate the greenish chloroform from the supernatant reagent. Any turbidity of the reagent, caused by precipitation of the leuco-base, may be removed by addition of glacial acetic acid. Should the reagent still have a green color, it may be removed by shaking with a little chloroform. *This reagent must be entirely colorless.*

Delicacy of the Test.—The relative delicacy of tests for blood by means of Teichmann's crystals, guaiac, and leuco-malachite green, or benzidine, is as follows:

Test	Positive in Dilution of
1. Teichmann's crystals	1:20,000
2. Guaiac test	1:500
3. Leuco-malachite green, or benzidine	1:100,000

Unfortunately a positive test is given by other substances than blood, for example, certain ferments (indirect oxidases) that collectively have the power of acting as indirect oxidizing agents in presence of hydrogen peroxide. Oxidizing ferments usually are present in animal liquids carrying leucocytes, such as urine, saliva and pus, but are destroyed by boiling. Sulphocyanates and ferrous salts also give positive tests with benzidine. So delicate are these two tests that a negative result makes it possible to come to a definite conclusion as to the absence of blood.

2. Biological Detection of Human Blood[1]

Injection of bacteria produces specific, bacteriolytic bodies and similarly injection of the blood of one animal species into an animal of a different species gives rise to specific, haemolytic and agglutinating bodies. Rabbit's blood, for example, injected repeatedly into a guinea pig, develops in the serum of such a guinea pig substances capable of agglutinating and dissolving red corpuscles of the rabbit, setting haemoglobin free and rendering the blood laky. Blood-serum of an animal, into which defibrinated blood, or blood-serum of a different animal species has been injected intravenously, subcutaneously or intraperitoneally, that is, into the peritoneal cavity, has the peculiar property of causing precipitation only in blood-serum of this particular animal species. Uhlenhuth,[2] Wassermann and Schütze,[3] and others have made independent experiments of this kind with blood-serum to find for forensic purposes a test, based upon this biological method, that shall differentiate human blood from the blood of every other animal species. Repeated injection of 10 cc. of defibrinated human blood, or human blood-serum free from cells, into a rabbit, either intraperitoneally or subcutaneously, yields a serum producing a heavy, cloudy precipitate in an aqueous solution of human blood. This coagulin is specific in action, producing a precipitate only in presence of human blood. Wassermann and Schütze tested the blood of 23 different animals, among which were mammals, birds and fishes, and obtained negative results with blood solutions from these very different animal species. By use of blood-serum it is possible to differentiate even old human blood, dried for many weeks, from the blood of other animals.

[1] This subject has been introduced for the sake of completeness. If such an investigation is for forensic purposes, the chemist will either decline to undertake it, or conduct the experiment with an associate who has had bacteriological and pathological experience.

[2] Deutsche med. Wochenschrift, 1911, No. 6 and Zeitschr. f. Medizinalbeamte, 1903, Nos. 5 and 6.

[3] Berliner Klinische Wochenscrift, 1911, No. 7.

To demonstrate the use of this method, Dieudonné[1] prepares 1 per cent. blood solutions, placing 2 cc. of the clear filtered solution in small test-tubes and adding an equal volume of double physiological salt solution (= 1.8 per cent. NaCl). Then add 6 drops of serum to each portion and place the tubes in an incubator at 37°. The serum of the rabbit, treated with human blood-serum, added to an aqueous solution of human blood, produced in a few minutes a distinct flocculent precipitate that gradually became more and more marked. As a check, test also with normal rabbit's serum which will cause no precipitate in a solution of human blood. Dieudonné found also that rabbit's serum, obtained after injecting human blood-serum, causes precipitates not only in human blood solutions but in human urine containing albumin, in an exudate from human pleura, and in peritoneal exudate. But precipitation in the case of human blood was much more marked than in these other tests. In his experiments Dieudonné used blood expressed from the placenta, repeatedly injecting it subcutaneously into rabbits in separate doses of 10 cc. and at intervals of 3–4 days. The animals were bled several days after the last injection and the blood was kept upon ice.

The antiserum used in detecting blood should above everything else be perfectly clear. To prepare such serum, use a sterile Berkefeld filter attached to a water-pump. The antiserum should be active in very dilute solution. Distinct turbidity should appear immediately in a solution diluted 1:1000, or in 1–2 minutes at latest. Sera must be of this high efficiency for practical use. Uhlenhuth has shown that the biological method of detecting blood is specific for human albumin. A necessary consequence of this fact is that the material should first of all be shown to be blood. The first question for the expert to answer in such an investigation must always be: "Is there any blood at all present?" If the answer is affirmative, the next question is: "Is it human or animal blood?" Consequently the material should first be examined for blood-stains by van Deen's ozone test, Teichmann's haemin test, and by the spectroscope. If the suspected stains are upon a hard surface, such as a knife, hatchet, gun-barrel, wood, stone, etc., they should be scraped off for the biological blood test and extracted for several hours in a test-tube with physiological salt solution (= 0.9 per cent. NaCl). First filter the extract through paper. If the filtrate is not clear, next use a Berkefeld filter.

PREPARATION OF REAGENTS

A. General Alkaloidal Reagents

A number of reagents, known as general alkaloidal reagents or as group reagents, produce when added to solutions of most salts of alkaloids insoluble or difficultly soluble precipitates that are characteristically colored, amorphous or crystalline. These reagents to be sure do not produce precipitates exclusively with solutions of alkaloidal salts. Certain of them, such as the chlorides of gold, platinum and mercury, phospho-molybdic acid and phospho-tungstic acid, react also with ammonia and many simple derivatives of ammonia as they do with alkaloids. This is due to the fact that alkaloids themselves are ammonia derivatives, that is, they are as a rule tertiary or secondary bases. Moreover,

[1] A. Dieudonné: Münchener med. Wochenschrift, 1911, 533.

proteins, albumoses, peptones, creatinine, and the purine bases, adenine, guanine, hypoxanthine and xanthine are precipitated by most of the alkaloidal reagents. For these reasons the general alkaloidal reagents may be designated as reagents for "nitrogen bases."

The general alkaloidal reagents are used especially to determine whether or not the material under examination contains an alkaloid or some other basic substance. When the ether extract of the aqueous alkaline solution, obtained in the Stas-Otto process, leaves a small residue, it should first be tested as to its behavior with general alkaloidal reagents before tests for individual alkaloids are made. To carry out these tests, dissolve a portion of the material in very dilute hydrochloric or sulphuric acid. Then filter the solution and distribute the filtrate in several test-tubes. Add to each test-portion a drop of the more delicate alkaloidal reagents. If an alkaloid or any other basic substance is present, all or nearly all tests will give distinct precipitates, or at least marked turbidity.

The following are the more important alkaloidal reagents:

Chlor-auric Acid.—An aqueous solution (1:30) which produces white, yellow or brown, amorphous or crystalline precipitates easily undergoing decomposition in part with separation of metallic gold.

Chloro-platinic Acid.—An aqueous solution (about 1:20) producing yellowish white to yellow precipitates that are usually granular and crystalline. Their composition is always analogous to that of ammonium chloro-platinate, $(H_4N)_2PtCl_6$.

Mercuric Chloride.—An aqueous solution (1:20) producing white to yellowish precipitates that are usually amorphous and gradually become crystalline.

Iodo-Potassium Iodide Solution (Wagner's Reagent).—A solution of 5 parts of iodine and 10 parts of potassium iodide in 100 parts of water producing precipitates that are brown and usually flocculent.

Potassium-Cadmium Iodide (Marmé's Reagent).—Dissolve 20 grams of potassium iodide in the same quantity of boiling water, add 10 grams of cadmium iodide and dilute this solution with water to 100 cc. With sulphuric acid solutions of most alkaloids, even when very dilute, Marmé's reagent produces white or yellowish, amorphous precipitates that after a while become crystalline and are soluble in excess of the reagent as well as in alcohol.

Silico-tungstic Acid.—This compound is $SiO_2.12WoO_3.2H_2O$. It forms well-defined, stable salts and in 5 per cent. aqueous solution is used as a very delicate alkaloidal reagent in the form of the free acid or as its alkali salt.[1]

Potassium-Mercuric Iodide (Mayer's Reagent).—A solution of 1.35 grams of mercuric chloride and 5 grams of potassium iodide in 100 cc. of water. With solutions of the hydrochlorides of most alkaloids it produces white or yellowish precipitates that are amorphous but after a while frequently become crystalline.

Potassium-Bismuthous Iodide (Dragendorff's Reagent).—According to Kraut,[2] this reagent is prepared by dissolving 80 grams of bismuth subnitrate in 200 cc.

[1] Crystallized silico-tungstic acid and its sodium salt may be procured from the firm of E. Merck, New York.

[2] K. Kraut: Bismuthous Iodide Compounds of Organic Bases. Ann. d. Chem., 210 (1881), 310; E. Jahns: Use of Potassium Bismuthous Iodide in the Preparation of Organic Bases. Arch. d. Pharm., 235 (1897), 151.

of nitric acid (sp. gr. 1.18 = 30 per cent. HNO_3) and pouring this solution into a concentrated solution of 272 grams of potassium iodide in a little water. When potassium nitrate has crystallized out, decant the solution after several days and dilute with water to 1 liter. This solution should not be exposed to light!

With solutions of sulphates of many alkaloids, potassium-bismuthous iodide produces beautiful, orange-red precipitates that are usually amorphous. If these precipitates are shaken with sodium hydroxide or carbonate solution, alkaloids usually can be recovered unchanged and frequently nearly quantitatively.

Potassium-Zinc Iodide.—Dissolve 10 grams of zinc iodide and 20 grams of potassium iodide in 100 cc. of water.

Phospho-molybdic Acid (Sonnenschein's Reagent).—(a) Saturate an aqueous solution of sodium carbonate with pure molybdic acid, adding 1 part of crystallized disodium phosphate ($Na_2HPO_4.12H_2O$) to 5 parts of the acid. Then evaporate to dryness, fuse the residue in a porcelain crucible and dissolve the melt when cold in water. Prepare 10 parts of solution from 1 part of residue. Add sufficient nitric acid to the filtered solution to produce a golden-yellow color.

(b) If free molybdic acid is not available, ammonium molybdate solution used in detecting phosphoric acid may be used by precipitating it completely at about 40° with sodium phosphate solution. The yellow precipitate is well washed, suspended in water, and warmed with a concentrated sodium carbonate solution until completely dissolved. Evaporate this solution to dryness, ignite the residue until all ammonia is expelled, moisten if reduction has taken place (blue to black color) with nitric acid and ignite again. Dissolve this residue in hot water with addition of nitric acid so that it is strongly acid. Prepare 10 parts of solution from 1 part of residue. The golden-yellow solution should be kept from exposure to ammonia vapor.

With solutions of sulphates of most alkaloids, phospho-molybdic acid produces amorphous precipitates having a yellow color. Frequently as a result of reduction of molybdic acid to molybdic oxide these precipitates after a while take on a greenish to bluish color.

Phospho-tungstic Acid (Scheibler's Reagent).—Dissolve 1 part of commercial sodium tungstate in 3 parts of water and add 0.5 part of 25 per cent. officinal phosphoric acid; or dissolve 100 grams of commercial sodium tungstate and 70 grams of disodium phosphate in 500 grams of water and acidify the solution with nitric acid. With solutions of alkaloidal salts phospho-tungstic acid usually produces amorphous, flocculent precipitates. With regard to the preparation of crystallized phospho-tungstic acid, consult Drechsel[1] and Winterstein.[2]

For use in colorimetric determinations of adrenaline and uric acid, prepare phospho-tungstic acid solution by heating 100 grams of sodium tungstate to boiling under a reflux with 80 cc. of 85 per cent. phosphoric acid and 750 cc. of water, keeping at a boil for 4 hours and diluting when cold to 1 liter with water. Preserve this reagent in small, dark bottles.

[1] E. Drechsel: Simple Methods for the Preparation of Some Complex Inorganic Acids. Ber. d. Deutsch. chem. Ges. 20 (1887), 1452.

[2] E. Winterstein: Preparation of Pure Phospho-tungstic Acid. Chem. Ztg., 1898, p. 539.

Tannic Acid Solution.—A freshly prepared 5 per cent. aqueous solution of tannic acid produces with solutions of alkaloidal salts white or yellowish, flocculent precipitates that partially dissolve in hydrochloric acid. Alkaloids may be recovered partially unchanged by treating these tannic acid precipitates with lead or zinc carbonate, evaporating and extracting the residue with ether, alcohol or chloroform.

Picric Acid.—A saturated, aqueous solution of picric acid produces yellow, crystalline precipitates, or those that are amorphous and soon become crystalline.

Picrolonic Acid.—This reagent is 0.1 n-alcoholic solution, containing in 1 liter of alcohol 0.1 gram of $C_{10}H_8N_4O_5$ = 26.4 grams of solid picrolonic acid. With most alkaloids this solution produces salts called picrolonates that are difficultly soluble, crystalline and have a yellow to red color. Picrolonic acid[1] behaves toward alkaloids and other bases as a monobasic acid.

B. Special Alkaloidal and Other Reagents

Bougault's Reagent.—Dissolve 20 grams of sodium hypophosphite in 20 cc. of water and then add 200 cc. of hydrochloric acid (sp. gr. 1.17 = 34 per cent.). The clear solution is decanted from the sodium chloride that separates out.

Bromo-Potassium Bromide.—A solution containing 1 gram of bromine and 2 grams of potassium bromide in 20 cc. of water. This reagent when kept easily loses bromine becoming less active or entirely inactive (R. Eder, see page 179).

Bromine-Hydrochloric Acid.—Shake concentrated hydrochloric acid vigorously with excess of bromine so that a saturated solution is produced.

Denigès Reagent.—Dissolve 5 grams of yellow mercuric oxide in a mixture of 100 cc. of water and 20 cc. of sulphuric acid while still warm.

Diazonium Reagent.—This reagent should be kept as two separate solutions, namely: **Solution I,** containing 0.5 gram of sulphanilic acid and 5 grams of hydrochloric acid in 100 grams of water; and **Solution II,** a 0.5 per cent. solution of sodium nitrite. The reagent is prepared by mixing 10 cc. of Solution I with 2 drops of Solution II.

p-Dimethyl-amino-benzaldehyde-Sulphuric Acid.—Dissolve 2 grams of p-dimethyl-amino-benzaldehyde, $(CH_3)_2N.C_6H_4.CHO$, in 6 grams of concentrated sulphuric acid and add to the solution 0.4 gram of water. This yellowish brown solution will keep several weeks (Wasicky, see page 178).

Erdmann's Reagent.—Sulphuric acid containing nitric acid, prepared by adding to 20 cc. of pure concentrated sulphuric acid 10 drops of a mixture of 10 drops of concentated nitric acid and 100 cc. of water.

Froehde's Reagent.—A solution of molybdic acid in sulphuric acid, prepared by heating gently and dissolving 5 mg. of molybdic acid or sodium molybdate in 1 cc. of pure concentrated sulphuric acid. The solution which should be colorless does not keep long.

Concentrated Froehde's reagent contains 0.01 gram of molybdic acid, or its sodium salt, in 1 cc. of concentrated sulphuric acid.

[1] L. Knorr: Amido-ethyl Alcohol. Ber. d. Deutsch. chem. Ges., 30 (1897), 909; H. Matthes and O. Rammstedt: Use of Picrolonic Acid in the Quantitative Estimation of Certain Alkaloids. Zeitschr. f. analyt. Chem., 46,565; Arch. d. Pharm. 245 (1907), 112.

Fehling's Solution.—This reagent is prepared from 2 separate solutions, one of copper sulphate and the other of alkaline Rochelle salt:

1. The copper sulphate solution contains 34.64 grams of pure crystallized copper sulphate, $CuSO_4.5H_2O$, in 500 cc.

2. The alkaline Rochelle salt solution is prepared by dissolving 173 grams of Rochelle salt, $NaKC_4H_4O_6.4H_2O$, and 50 grams of sodium hydroxide in hot water and diluting this solution when cold to 500 cc. with water.

Fehling's solution should be prepared as needed by mixing equal volumes of these two solutions. If this reagent has been prepared and kept for some time, it should always be tested before being used. If when heated it gives a precipitate of cuprous oxide, it should be rejected.

Formaldehyde-Sulphuric Acid.—Marquis reagent,[1] prepared as needed by mixing 2–3 drops of 40 per cent. formaldehyde solution with 3 cc. of pure concentrated sulphuric acid.

Fuchsine-Sulphurous Acid.—According to G. Denigès, this reagent is prepared by mixing a solution of 1 gram of fuchsine in 1 liter of water with 50 cc. of saturated sodium bisulphite solution and acidifying with 1 cc. of concentrated sulphuric acid.

Fuchsin-Sulphurous-Hydrochloric Acid.—A reagent for formaldehyde prepared according to Grosse-Bohle. Dissolve a gram of pure acetate or hydrochloride of rosaniline in about 500 cc. of water, add an aqueous solution of 25 grams of crystallized sodium sulphite together with 15 cc. of hydrochloric acid (sp. gr. 1.124 = 25 per cent.), and then bring the volume with water to 1 liter. This mixture slowly loses color but may be used after a few minutes. If allowed to stand in a closed bottle, it keeps for a long time.

Günzburg's Reagent.—A solution of phloroglucinol and vanilline prepared by dissolving 1 part of phloroglucinol and 1 part of vanilline in 30 parts of alcohol. This reagent is used for detection of free mineral acids, especially free hydrochloric acid. Free organic acids and acid phosphates do not react with Günzburg's reagent.

Hünefeld's Solution.—This reagent is prepared by adding 25 cc. of alcohol, 5 cc. of chloroform and 1.5 cc. of glacial acetic acid to 15 cc. of rather old oil of turpentine, that has been exposed for some time to air and light but by itself should not turn guaiac tincture blue, or 15 cc. of 3–4 per cent. acid-free hydrogen peroxide. This solution is used for detection of blood.

Iodic Acid Solution.—A 10 per cent. aqueous solution of iodic acid, HIO_3.

Leuco-Malachite Green Reagent.—Preparation of this reagent, used for the detection of blood according to Adler, is described on page 636.

Liebermann's Reagent.—A solution of 0.6 gram of potassium nitrite in 10 grams of concentrated sulphuric acid.

Magnesia Mixture.—Dissolve 11 grams of crystallized magnesium chloride, $MgCl_2.6H_2O$, and 14 grams of ammonium chloride in 130 cc. of water and add 70 grams of ammonia (sp. gr. 0.96 = 10 per cent. NH_3). This mixture should be clear. It is used for detection of arsenic acid and phosphoric acid.

[1] R. Kobert: Detection of Morphine and Derivatives. Apoth. Ztg., 14 (1899), 259; also H. Linke: Behavior of Certain Organic Bodies, particularly Alkaloids, toward Sulphuric Acid containing Formaldehyde. Ber. d. Deutsch. pharm. Ges., 11 (1901), 258.

Mandelin's Reagent.—Dissolve 1 part of finely ground ammonium vanadate cold in 200 parts of pure concentrated sulphuric acid.

Mecke's Reagent.[1]—Dissolve 1 gram of selenious acid in 200 grams of pure concentrated sulphuric acid.

Millon's Reagent.—Dissolve 1 part of metallic mercury with cooling in 1 part of cold fuming nitric acid, or in 1 part of nitric acid (sp. gr. 1.4 = 65 per cent.) with gentle warming, and then dilute the solution obtained in either manner with 2 parts of distilled water. Allow the solution to settle and decant the clear liquid from any crystalline precipitate that has formed.

Morphine-Sulphuric Acid.—Jorissen's reagent for formaldehyde prepared by dissolving 0.2 gram of morphine hydrochloride in 10 cc. of cold, pure concentrated sulphuric acid. This reagent does not keep and consequently should be freshly made just before used.

Naphthol Reagent.—Used for detection and colorimetric estimation of nitrous acid.[2] Dissolve 2 grams of chemically pure sodium naphthionate, $C_{10}H_6(NH_2)$-(SO_3Na) = 1, 4, and 1 gram of purest β-naphthol, $C_{10}H_7.OH$, with vigorous shaking in 200 cc. of water and then filter. The colorless solution obtained may be kept in dark bottles for some time without actual change.

Nessler's Reagent.—The solution contains 10 grams of mercuric iodide, HgI_2, 5 grams of potassium iodide, 20 grams of sodium hydroxide and 100 cc. of water. Triturate mercuric iodide in a porcelain mortar with a little water, then rinse into a flask and add potassium iodide. Dissolve sodium hydroxide in the remainder of the water and mix when cold with the solution of mercuric and potassium iodide. Preserve the clear settled solution in the dark in small bottles.

Phloroglucinol Reagent.—Dissolve 1 gram of pure phloroglucinol in 100 cc. of 10 per cent. sodium hydroxide solution. This reagent is used for formaldehyde. Since it does not keep very long, it should be freshly prepared just before used, especially for colorimetric estimations of formaldehyde.

Ringer's Solution.—The solution isotonic for frogs contains in 1 liter 0.1 gram of sodium bicarbonate, 0.1 gram of dry calcium chloride, 0.075 gram of potassium chloride and 6.0 grams of sodium chloride. Sodium bicarbonate should first be completely dissolved in distilled water before calcium chloride is added.

Rosenthaler's Reagent.—A specific reagent for opium alkaloids, prepared by dissolving 1 gram of potassium arsenate in 100 grams of concentrated sulphuric acid.

Schaer's Alkaloid Reagent.—Carefully mix 1 volume of Merck's pure 30 per cent. perhydrol with 10 volumes of pure concentrated sulphuric acid. This reagent should always be freshly prepared.

Sonnenschein's Reagent.—This reagent used for strychnine is prepared by dissolving 0.1 gram of cerium oxide in 10 cc. of water and adding gradually 50 cc. of concentrated sulphuric acid.

Uranium Nitrate Reagent.—Aloy's reagent for morphine and phenols prepared by dissolving 10 grams of uranyl nitrate, sulphate, acetate or oxalate in 60 cc. of

[1] E. Mecke: A New Reagent for Alkaloids. Zeitschr. f. öffentl. Chem., 5 (1899), 351.

[2] E. Riegler: A very delicate Reaction for Nitrites and their Quantitative Estimation colorimetrically. Zeitschr. f. analyt. Chemie, 36 (1897), 377.

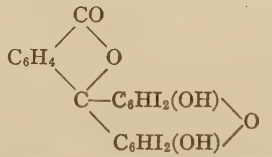
water, adding dilute ammonia until turbidity begins to appear, filtering and diluting the filtrate with water to 100 cc.

Stannous Chloride Solution.—Triturate 5 parts of crystallized stannous chloride with 1 part of hydrochloric acid until the mass is uniform and then saturate with dry hydrochloric acid gas. After the solution thus obtained has settled, filter through asbestos. It is a pale yellowish liquid that is refractive, fumes strongly, and has a specific gravity of at least 1.9. This solution is used for the detection of arsenic (Bettendorff's Test for Arsenic).

For better preservation, stannous chloride solution should be kept in small glass-stoppered bottles that are completely filled.

C. The Indicator Iodeosine

Iodeosine or Erythrosine.—This compound is tetriodo-fluoresceine having the formula $C_{20}H_8I_4O_5$. Since it is formed by iodination of fluoresceine, it has the following structure:



The commercial product usually contains as impurities small quantities of substances that are almost insoluble in ether. To purify[1] commercial iodeosine, dissolve in aqueous ether and then extract from the filtered ether solution by means of dilute sodium hydroxide solution. Strong sodium hydroxide solution will precipitate from this aqueous alkaline solution the sodium salt of iodeosine which may be filtered, washed with cold alcohol, and after recrystallization from hot alcohol obtained in well-formed, nearly rectangular plates having a superficial green color. Hydrochloric acid will then precipitate pure iodeosine from the aqueous solution of its sodium salt. Pure iodeosine dried at 120° is lighter in color than the commercial product. It is almost insoluble in absolute ether, benzene and chloroform; more easily soluble in acetone, alcohol and aqueous ether. The shade of color in aqueous solutions of caustic alkalies of the purified dyestuff is yellower than that of the crude product. Iodeosine is a scarlet-red, crystalline powder soluble in alcohol with deep red and in ether with yellow-red color. Iodeosine should be insoluble in water rendered acid with a trace of hydrochloric acid. To prepare the indicator "Iodeosine Solution," dissolve 1 gram of iodeosine in 500 grams of alcohol.

D. Curcurmine and Curcurmine Paper

Curcurmine and Curcurmine Paper.[2]—Curcurmine is prepared in the following manner: First, extract 30 grams of finely ground curcurma root (Curcurma longa),

[1] Fr. Mylius and F. Foerster: Estimation of small Quantities of Alkali and Recognition of the Neutrality of Water. Ber. d. Deutsch. chem. Ges. 24 (1891), 1482.

[2] This method of preparation is according to the official "Directions for the Chemical Examination of Wine" of December 9th, 1920.

dried at 100°, in a Soxhlet extractor with petroleum ether for 4 hours. The dried powder freed in this manner from fat is then extracted in the same apparatus for 8–10 hours with 100 cc. of boiling benzene. A glycerol bath heated at 115–120° may be used to boil the benzene. This benzene solution upon cooling deposits within 12 hours curcurmine used in preparing curcurmine paper. This paper is prepared by saturating white filter paper once with a solution of 0.1 gram of curcurmine in 100 cc. of alcohol (90 per cent. by volume). Dried curcurmine paper is protected from light in tightly closed bottles.

APPENDIX

Meyer-Overton[1] Theory of Narcosis

In living cells occur oil-like substances soluble in boiling alcohol and in ether. They are called "lipoids." These lipoid substances in cells, according to Overton, are composed of lecithins, abundant in cell stromata, and cholesterol. Stromata of red blood-corpuscles are said to consist one-third of lipoids and two-thirds of protein. As the result of an extended study of narcotization of animals and plants, Meyer and Overton deduced the theory that all indifferent substances capable of dissolving fat and fat-like substances (lipoids) exert narcotic action upon living protoplasm, provided they can diffuse into it. Since all animal and plant cells are saturated with water, obviously a substance must be soluble in water before it can find its way into cells. In order therefore for a narcotic to enter those parts of body cells containing lipoids, it must not only possess the power of dissolving fats and lipoids but at the same time be sufficiently soluble in water. The quantitative distribution of such a substance in the living body must therefore depend upon the ratio of its solvent affinity for fat (lipoid) and for water which is the distribution coefficient: $\frac{\text{Lipoid solubility}}{\text{Water solubility}}$! The chief requirement for manifestation by any substance of narcotic action, according to this theory, is solubility of fat together with solubility of water. Blood plasma in which the narcotic is dissolved bathes the body cells and they abstract the particular substance from the blood by the law of distribution according to the amount of lipoid substances they contain. Narcotic effect must be strongest in those cells in the chemical composition of which fat-substances and lipoids predominate and actually perform the cell function, preeminently nerve cells.

In their distribution throughout the organism, narcotics prefer those cells made up chiefly of fats and lipoids and they are stored in those organs composed of such cells. Experiments with chloroform, which is more abundant in red blood-corpuscles than in serum, have shown this to be the case, because it is held there by cholesterol and lecithin, the chloroform-soluble constituents of erythrocytes (Pohl, 1891). Ether, chloral hydrate and acetone exhibit the same unequal distribution between blood-corpuscles and plasma (Frantz, Archangelsky[2]). In the case of dog's blood, their experiments show that 0.03–0.05 per cent. of chloral hydrate must be present before narcosis can appear. When the blood contains 0.11–0.12 per cent. of chloral hydrate, respiration ceases. Distribution of chloral hydrate in the blood was not uniform but was mostly in the blood-corpuscles. In the initial stage of narcosis, chloral hydrate was relatively

[1] H. H. Meyer: Theory of Alcohol Narcosis. Archiv f. experim. Pathol. u. Pharmakol. 42, 109 (1899). E. Overton: Studies of Narcosis, Jena, 1901.

[2] C. Archangelsky: Distribution of Chloral Hydrate and Acetone in the Organism. Archiv f. experim. Pathol. u. Pharmakol., 46,347 (1901).

less in the brain than in the blood; and the percentage of chloral hydrate in the brain does not exceed that in the blood, until narcosis has been in progress for some time. On the other hand, the quantity of chloral hydrate in the liver and spleen is always far less than in the brain. Consequently the affinity of chloral hydrate appears to be specific for the central nervous system.

In the case of acetone, deep narcosis did not appear until the quantity in the blood reached 0.5 per cent. Most of the acetone was in the blood-corpuscles. The brain always contains somewhat more and the liver somewhat less acetone than the blood. This theory of narcosis that narcotics dissolve especially easily in the fat-like and lipoid-like constituents of protoplasm offers a simple explanation of these facts.

In the distribution of a substance between water and oil, according to Meyer and Overton, the more abundantly it is taken up by the latter, the better it acts as a narcotic. The greater therefore the quotient $\frac{\text{Oil}}{\text{Water}}$, the less need be the concentration of the narcotic solution in proximity to a cell, that is, in the blood. Consequently every lipoid-soluble substance has a critical concentration above which it exerts narcotic action, that is, arrests a definite activity of a particular cell, and below which either no narcosis appears or existing narcosis disappears. The assumption (Meyer, Overton), that the lower the critical concentration, the greater the distribution quotient $\frac{\text{Oil}}{\text{Water}}$, suggests the idea that narcosis always occurs when the cell-lipoids have absorbed the narcotizing substance up to a certain molecular concentration. The same idea may be expressed by saying that narcosis always occurs when the cell-lipoids have been saturated with the same number of narcotic molecules. It is immaterial whether they take these narcotic molecules from a solution of a strong narcotic of low concentration, or from a solution of a weak narcotic of high concentration. With certain limitations these statements hold. The conclusion should not be drawn that mere concentration of the narcotic in the lipoids as a fixed absolute magnitude, quite independent of the nature of the narcotic, determines the beginning of narcosis, for every narcotizing substance, according to its chemical constitution and affinities for the constituents of protoplasm, is more or less individual in its action upon cells.

When the reversible influence of differences in temperature upon the same substance is considered, the necessary connection existing between the coefficient of distribution and strength of narcosis becomes especially clear and convincing. Elevation of temperature increases the distribution-coefficient of some substances in accordance with individual differences of solubility, whereas it decreases that of other substances. Similarly the strength of narcotic action rises or falls. According to Meyer's experiments, the distribution-coefficient $\frac{\text{Oil}}{\text{Water}}$ is 0.053 at 3° for chloral hydrate but 0.236 at 30°, and the corresponding active dilutions, just causing paralysis of tadpoles, are $\frac{1}{50}$ n-chloral hydrate solution at 3° and $\frac{1}{250}$ n-chloral hydrate solution at 30°. Consequently tadpoles narcotized in $\frac{1}{250}$ n-chloral hydrate solution at 30° will revive, if the solution is cooled, and become narcotized once more if the solution is again warmed.

It may not be out of place to point out that the narcosis theory of Meyer and Overton merely states how narcotics reach the place where they act without explaining how they exert their action there.

This narcosis theory has met with objections from various quarters. Höber (Physical Chemistry of the Cell, IV Edition, page 457) has obtained experimental results from which he concludes that narcotics arrest certain changes of colloidal condition that accompany the process of irritation. Inasmuch as lipoids are markedly colloidal in character, a colloid theory of narcosis is possible.

Further emphasis may be placed upon the fact that the Meyer-Overton theory of alcohol narcosis is not applicable to every kind of narcosis. The mechanism of the action already mentioned holds only for chemically indifferent substances. In this sense substances having no relation to narcotics of the aliphatic series, such as nitrous oxide and carbon dioxide, also belong to the pharmacological alcohol group. These substances have a narcotic action and also dissolve lipoids, whereas the carbonates possess neither property.

On the other hand, alkaloids exhibit not only affinity for fats and lipoids, which they manifest in the free state, but affinities also for other cell constituents. Viewed from different angles, the diversity of the action of alkaloids upon the nervous system is bound up with the structure of the nerve cells.

The narcotic action of chloroform, as well as that of other narcotics of the alcohol group, is essentially reversible. Consequently, if the quantity of narcotic in the blood becomes less on account of elimination by the lungs or kidneys, or from decomposition, the lipoids return to the blood a part of what they hold and so their supply is depleted. For this reason narcosis manifests itself and then ceases.

During chloroform narcosis, certain parts of the nervous system, as well as deposits of fat well-supplied with blood, contain more chloroform than the other organs. This was shown to be the case from the distribution of chloroform in dogs after narcosis lasting 84 minutes (Meyer-Gottlieb):

Organ		Percentage of Chloroform
Medulla oblongata		0.085
Spinal cord		0.083
Liver		0.050
Kidneys		0.046
Spleen		0.031
Heart		0.039
Muscle		0.024
Fat	Omentum	0.068
	Around kidneys	0.132

The other indifferent narcotics behave in a similar manner during narcosis.

Constitution of Colchicein and Colchicin

The latest investigations of Windaus[1] have been so successful that they may be said to furnish a complete explanation of the constitution of colchicin. As

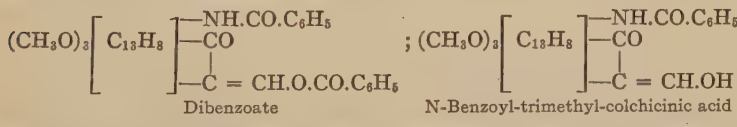
[1] A. Windaus and H. Schiele: Constitution of Colchicin. Nachrichten der K. Gesellschaft der Wissenschaften zu Göttingen. Mathematisch-physikalische Klasse, 1923. Pages 17–36.

stated elsewhere (see Colchicin, page 115), nearly neutral colchicin warmed with 0.5 per cent. hydrochloric acid loses 1 molecule of methyl alcohol and becomes colchicein. The latter is a weak acid and by the very characteristic test it gives with ferric chloride shows that it is actually different from colchicin. If colchicein is then heated with 15 per cent. hydrochloric acid, it loses 1 molecule of acetic acid and passes into trimethyl-colchicinic acid:

$$\underset{\text{Colchicin}}{C_{22}H_{25}O_6N} + H_2O = \underset{\text{Colchicein}}{C_{21}H_{23}O_6N} + \underset{\text{Methyl alcohol}}{CH_3OH}$$

$$\underset{\text{Colchicein}}{C_{21}H_{23}O_6N} + H_2O = \underset{\text{Trimethyl-colchicinic acid}}{C_{19}H_{21}O_5N} + \underset{\text{Acetic acid}}{CH_3.COOH}$$

Trimethyl-colchicinic acid forms salts both with bases and acids and contains a primary amino-group as shown by its behavior on exhaustive methylation. The acetyl-group replaces a hydrogen atom of this amino-group (—$NH.CO.CH_3$) in colchicin. Three methoxyl-groups are still in trimethyl-colchicinic acid as in colchicein, since by Zeisel's method 3 molecules of methyl iodide are formed by heating with concentrated hydriodic acid. Although these experiments afford certain proof of the methoxyl-groups and of the N-acetyl-group, various facts are not in agreement with the assumption made by Zeisel that colchicein contains a carboxyl-group. In the first place the fine color reaction given by colchicein with ferric chloride is one obtained with phenols and enols but not with carboxylic acids. The chemical behavior of aldo-enols and keto-enols points to the membership of colchicein in this class of compounds. Windaus was the first to call attention to this fact. The ease with which the methyl ether of colchicein, and also of colchicin, is saponified, especially indicates presence of an enol rather than of a phenol. The idea that the enol form of a β-diketone, or of a β-keto-aldehyde, is present in colchicein is further confirmed by the behavior of trimethyl-colchicinic acid on acylation, for example, on benzoylation with benzoyl chloride and sodium hydroxide, or in pyridine solution with benzoyl chloride. A dibenzoyl-derivative no longer giving the ferric chloride test is formed. Apparently this compound contains one benzoyl-group on the nitrogen atom and the other combined with oxygen of the enol-group. By careful saponification, this dibenzoyl-compound may be made to lose the benzoyl-group on the oxygen atom and give N-benzoyl-trimethyl-colchicinic acid. The latter has acid properties and gives a fine dark-green color reaction with ferric chloride:



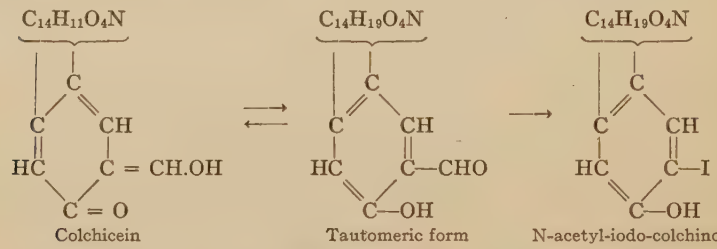
The fact discovered by Windaus that trimethyl-colchicinic acid dissolved in pyridine gives upon treatment with benzene-sulpho-chloride two stereo-isomeric N-benzene-sulphone-trimethyl-colchicinic acids agrees best with the conception that this acid is an oxymethylene compound. On the other hand, if trimethyl-colchicinic acid is a carboxylic acid, as Zeisel supposes it to be, the carboxyl-group would have to react with the sulphonic acid group and form a mixed acid anhydride. In that case appearance of cis-trans isomeric acid derivatives would

be unintelligible. The fifth oxygen atom of colchicein, on the basis of these experimental results, may be correctly regarded as enol-oxygen. Upon the assumption that colchicein and trimethyl-colchicinic acid are enols of β-diketones, or β-ketone aldehydes, the sixth oxygen atom must belong to a carbonyl-group. The behavior of colchicein when heated with glacial acetic acid and bromine, resulting in the replacement of three hydrogen atoms by bromine and at the same time in the addition of an atom of oxygen with formation of the finely crystallizing tribromo-colchicinic acid, is in favor of the enol-form of a β-ketone aldehyde, since formation of a carboxylic acid through addition of an atom of oxygen can come about only from an aldehyde:

$$\underset{\text{Colchicein}}{C_{21}H_{23}O_6N} + O + 3Br_2 = \underset{\text{Tribromo-colchicinic acid}}{C_{21}H_{20}O_7NBr_3} + 3HBr$$

A second proof offered by Windaus of the presence of an aldehyde, or oxymethylene-group, in the colchicein molecule is the reaction due to the action of iodo-potassium iodide solution upon colchicein in a faintly alkaline solution. N-acetyl-iodo-colchinol, $C_{20}H_{22}O_5NI$, formed by this reaction, differs from colchicein in containing iodine in place of the aldehyde-group (—CHO). N-acetyl-iodo-colchinol does not give the ferric chloride reaction of colchicein, nor is this reaction given if iodine is replaced by hydrogen.

This striking reaction is analogous to that given by oxymethylene-camphor, bromine and potassium hydroxide, whereby bromo-camphor is obtained (Claisen) N-acetyl-iodo-colchinol certainly is no longer a ketone. In behavior it is more like a phenol, for it is soluble in dilute potassium hydroxide solution and is again set free from this solution by carbon dioxide. Furthermore it is easily acetylated and methylated. N-acetyl-iodo-colchinol may be regarded as a complex derivative of an ortho-iodo-phenol. The transition of colchicein into N-acetyl-iodo-colchinol is shown by the following structural formulae:

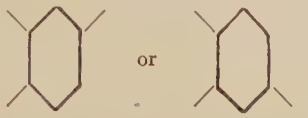


Colchicein, according to this conception, is closely related to the aromatic ortho-oxy-aldehydes and its chemical behavior is in complete agreement with this idea. Concentrated hydrochloric acid changes the slightly colored alcoholic or aqueous solutions of colchicin and its derivatives to a deep yellow. This color change is due to formation of addition products that may be isolated as such to some extent. Trimethyl-colchicinic acid, for example, gives from a saturated alcoholic solution a difficultly soluble, deep yellow dihydrochloride. Potassium hydroxide solution also colors colchicin and its derivatives deep yellow. The ortho-oxy-aldehyde, salicylic aldehyde, $C_6H_4(OH)(CHO)$, gives similar colorations. Alcohol containing hydrochloric acid, or glacial acetic acid,

dissolves it with yellow color. Aqueous potassium hydroxide solution also produces a deep yellow coloration. Aromatic ortho-oxy-aldehydes also behave like colchicein when treated with iodine in presence of potassium hydroxide.

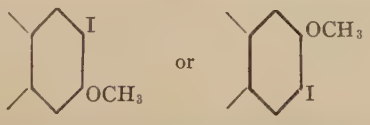
Three Different Six-carbon Rings in Colchicin

Vigorous oxidation of colchicin, colchicein, trimethyl-colchicinic acid, or N-acetyl-iodo-colchinol with hot potassium permanganate solution gives in each instance the same oxidation product, namely, 3,4,5-trimethoxy-1,2-phthalic acid. This proves the presence of trimethoxy-benzene ring I. The course of

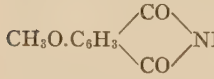


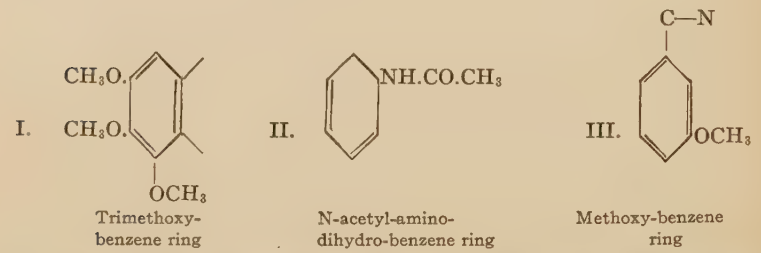
the reaction, however, is different, if before oxidation the methoxy-groups are saponified and ammonia is also split out by fusion with potassium hydroxide. Oxidation by means of potassium permanganate now gives different benzene carboxylic acids, in the main terephthalic acid, $C_6H_4(COOH)_2(1:4)$, and trimellitic acid, $C_6H_3(COOH)_3(1:2:4)$. From this result it is probable that in addition to the trimethoxy-benzene ring a second partially hydrogenized benzene ring with the adjacent grouping is also present in colchicin. Finally proof has been furnished that the colchicin molecule further contains a third benzene ring that is different from the other two.

By means of diazo-methane, or dimethyl-sulphate, N-acetyl-iodo-colchinol as a phenol may be converted into its methyl ether, the newly formed methoxyl-group of which is very stable toward hot nitric acid. If this n-acetyl-iodo-colchinol methyl ether is oxidized first with nitric acid and then with hot potassium permanganate solution, the trimethyoxy-benzene ring I and the dihydroamino-benzene ring II (see below) are destroyed, whereas a third ring rendered very resistant to oxidation by methylation of the phenolic hydroxyl remains intact. Among the products of the reaction, iodo-methoxy-o-phthalic acid, $C_6H_2I(OCH_3)(COOH)_2$ has been found. In this compound the methoxy-group occupies the 4-position, for it may be reduced by means of zinc dust and acetic acid to 4-methoxy-phthalic acid. The formula of this iodo-methoxy-phth-



alic acid is probably that of a 4-methoxy-5-iodo-benzene-1, 2-dicarboxylic acid, originating from a third ring present in colchicin, as shown in the adajcent groupings. A result obtained by oxidation has afforded an explanation with regard to the position of the nitrogen atom in ring III of the colchicin molecule. N-acetyl-iodo-colchinol methyl ether yields upon reduction with zinc dust N-acetyl-colchinol methyl ether. Vigorous treatment of the latter with concentrated hydrochloric acid causes saponification of the three methoxyl-groups in ring I but does not affect the methoxyl-group in ring III. If this action is followed by

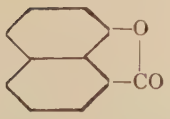
oxidation with chromic anhydride and sulphuric acid, rings I and II are destroyed and the product obtained from ring III is 4-methoxy-o-phthalimide,  The three rings alluded to above are the following:



N-acetyl-colchinol methyl ether, containing 21 carbon atoms, cannot be made up of three independent rings, since such a structure would require 24 carbon atoms. Several carbon atoms must function in more than one ring, that is, all three rings must be condensed with one another. Colchicin therefore must be an anthracene or phenanthrene derivative! A complete explanation of the constitution of colchicin has been made possible because of the insight into the nature of this molecule afforded by certain reactions of decomposition as a result of which colchicin has been converted, on the one hand, into dihydro-naphthalene and on the other hand into tetra-methoxy-methyl-phenanthrene and methyl-phenanthrene.

Careful oxidation of N-benzoyl-colchicinic acid by means of potassium permanganate gives N-benzoyl-colchide, $C_{23}H_{23}O_6$ (VII), and N-benzoyl-colchicinic anhydride, $C_{23}H_{21}O_7N$ (VI), these two compounds containing three less carbon atoms than the original compound! They do not respond to the ferric chloride test, nor do they contain the specific groups of colchicein. However, it is probable that they still contain the three methoxy-groups of ring I, since upon further oxidation they give 3,4,5-trimethoxy-o-phthalic acid. If N-benzoyl-colchide is heated dry, it breaks down into benzamide and a compound, $C_{16}H_{16}O_5$, trimethoxy-homonaphthide (VIII). The latter was established as a naphthalene derivative.

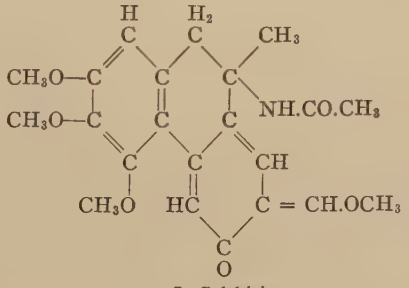
The second oxidation product, N-benzoyl-colchicinic anhydride, threw further light upon the problem. This compound is to be regarded as the anhydride of a dicarboxylic acid. The compound itself, or its tetrahydro-derivative obtained by means of zinc dust and glacial acetic acid, when heated to boiling with concentrated hydriodic acid, loses 3 molecules of methyl iodide, 1 molecule of carbon dioxide, and 1 molecule of water. The product is a neutral anhydride, $C_{19}H_{17}O_5N$, the formation of which can be brought about only through the interaction of the remaining carboxyl-group and the phenolic hydroxyl resulting from saponification. Since the carboxyl-groups and methoxyl-groups are in different rings, this anhydride formation necessitates a bridge-like union of the two rings. Such a union, however, never occurs in the case of the naphthalene compounds, unless the two groups occupy the peri-positions (Sachs):



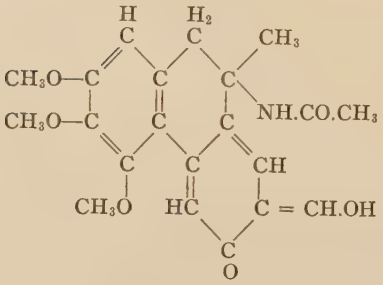
Inasmuch as the two carboxyl-groups represent the remnants of ring III. that has been destroyed by oxidation, this ring must have been attached in a manner requiring in colchicine the existence of a phenanthrene and not of an anthracene ring. This idea has been convincingly established by the following reaction. N-acetyl-iodo-colchinol methyl ether (IV′), containing 4 methoxyl-groups three of which are in ring I and the fourth in ring III, has been converted by way of halogen-free N-acetyl-colchinol methyl ether (IV) into the free amine, colchinol methyl ether (V) containing 4 methoxyl-groups and a primary amino-

group. This amino-group can be removed by Hofmann's cleavage-reaction as trimethyl-amine. An entirely neutral compound, having the formula $C_{19}H_{20}O_4$, was obtained in this way. It was found to contain 4 methoxyl-groups. Consequently this compound is tetra-methoxy-methyl-phenanthrene (IX), having the formula $(CH_3O)_4(C_{15}H_8)$. The latter heated with concentrated hydriodic acid loses four methoxyl-groups as methyl iodide. The tetrahydric phenol first formed yields, when reduced by distillation with zinc dust, an oil that is brilliantly fluorescent. Repeated fractional sublimation of this product gives a hydrocarbon, $C_{15}H_{12}$, melting at 88° and resembling phenanthrene in its properties. This compound must be 9-methyl-phenanthrene (X).

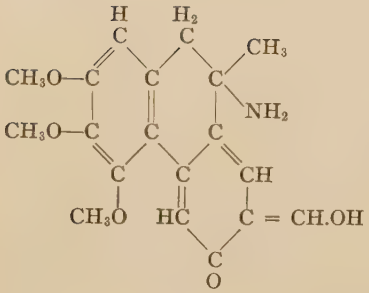
Any structural formula that is to represent colchicin must have regard for the fact that the molecule contains a reactive methylene-group, for Zeisel succeeded in oxidizing colchicin by means of chromic anhydride to oxy-colchicin, $C_{22}H_{23}O_7N$ (XI), a procedure corresponding in every way to the oxidation of tetraline to α-tetralone. Windaus and Schiele have embodied all these facts in the following structural formulae which seem to offer the most satisfactory explanation of the constitution of colchicin and its decomposition products:



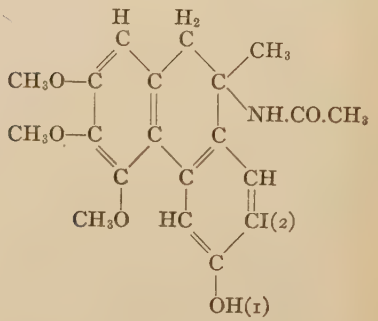
I. Colchicin



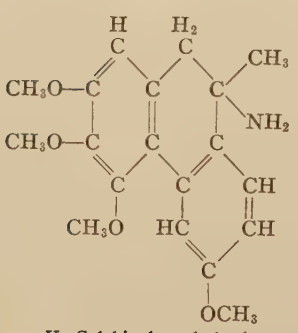
II. Colchicein



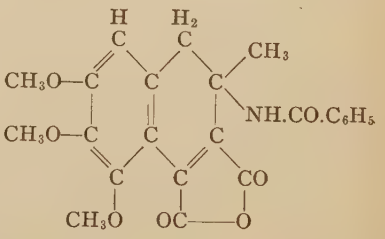
III. Trimethyl-colchicinic acid



IV. N-acetyl-iodo-colchinol[1]

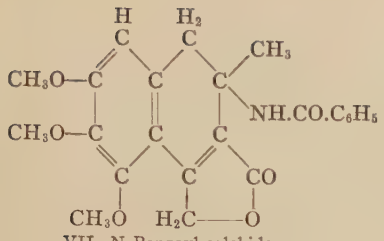


V. Colchinol methyl ether

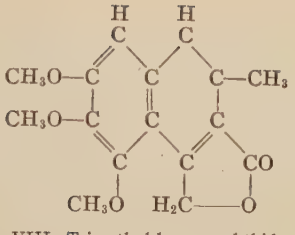


VI. N-Benzoyl-colchicinic anhydride

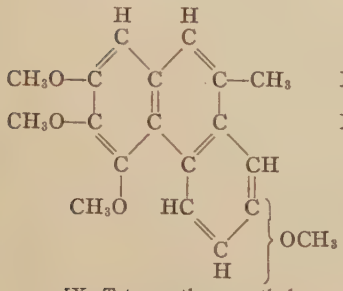
[1] If the hydrogen atom of N-acetyl-iodo-colchinol (IV), that is marked (1), is replaced by methyl, the product is N-acetyl-iodo-colchinol methyl ether (IV′). If hydrogen is substituted for the iodine atom marked (2) in this ether, the formula of N-acetyl-colchinol methyl ether is obtained.



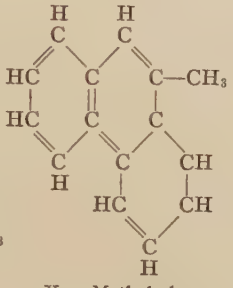
VII. N-Benzoyl-colchide



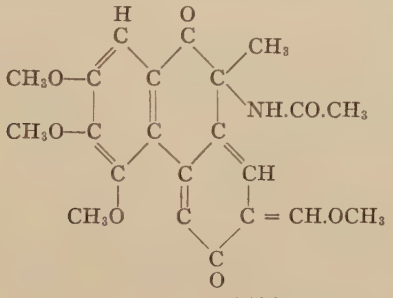
VIII. Trimethyl-homonaphthide



IX. Tetra-methoxy-methyl-phenanthrene



X. 9-Methyl phenanthrene



XI. Oxy-colchicine

Alkaloids of Lobelia Inflata

(See the Article "Lobeline" on Page 398)

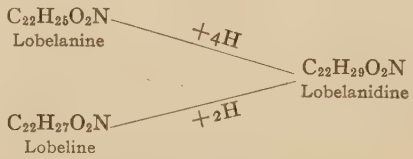
Wieland[1] obtained from the leaf of Lobelia inflata four well-characterized alkaloids that melt sharply and crystallize well, namely:

Lobeline, $C_{22}H_{27}O_2N$,[2] prisms from alcohol, melting at 129–130°,
Lobelanine, $C_{22}H_{25}O_2N$, needles from ether, melting at 99°,
Lobelanidine, $C_{22}H_{29}O_2N$, prisms from alcohol, melting at 150°,
Lobelidine, $C_{20}H_{25}O_2N$, small prisms from alcohol, melting at 106°.

[1] Private communication from Professor H. Wieland.

[2] The formula $C_{23}H_{29}O_2N$ formerly given to crystalline lobeline has been changed to $C_{22}H_{27}O_2N$.

All these Lobelia alkaloids are monacid bases. Their salts with one equivalent of acid, particularly with hydrochloric, hydrobromic and nitric acid, crystallize well. Upon hydrolysis, to some extent even when boiled with water, all with the exception of lobelanidine undergo cleavage of acetophenone, $C_6H_5.CO.CH_3$. There is no question apparently as to their close chemical relationship. One reason among others for this view is the fact that lobelanine and lobeline may be converted into lobelanidine by reduction:



Pharmacological Action.—Crystallized lobeline, $C_{22}H_{27}O_2N$, according to the researches of Wieland,[1] produces decided stimulation of the respiratory centre but does not give rise (see page 398) to a series of actions hitherto regarded as characteristic of lobeline, for example, stimulation of the emesis centre. Pure lobeline also exhibits no action like that of atropine upon the vagus endings in the bronchial muscles and heart. In the case of lobeline solutions of very high concentration, Wieland has shown that they act injuriously upon the heart muscle

Lobeline exerts an influence upon paralysis of the respiratory centre arising from different poisons producing such paralysis. For example, paralysis caused by morphine can be very easily repressed, that by urethane less easily, and an intoxication from chloral hydrate with relative difficulty. The more severe the paralysis of the respiratory centre is, the larger the quantity, that is, the higher the concentration, of lobeline must be to produce stimulation of the respiratory centre.

Lobeline also exhibits specific action upon the circulation of the blood. Respiratory action and the action of the circulation, according to Wieland, stand in very close relationship. The most striking symptom of the latter effect of lobeline, noted in rabbits after intravenous injection, is the appearance of a marked decrease of blood-pressure distinctly evident after 0.25 mg. of lobeline. But this lowering of blood-pressure is only of short duration and sometimes is accompanied by temporary elevation of blood-pressure lasting for 1–3 minutes.

Lobelanine, also called base B, as well as lobelidine, also stimulates respiration but only about half as strongly as lobeline. Their other physiological actions resemble those of the principle alkaloid of Lobelia inflata, lobeline.

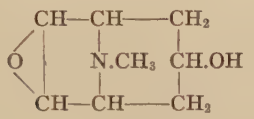
Because of the property it possesses of stimulating respiration, lobeline has been put to practical use in instances of respiratory paralysis arising during narcosis (Hellwig[2]).

[1] H. Wieland and R. Meyer: Pharmacological Investigations of the Respiratory Centre II, Archiv f. exper. Pathol. u. Pharmakol. 92 (1922), 196.

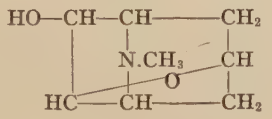
[2] Hellwig: Lobeline in Respiratory Paralysis during Narcosis. Zentralbl. f. Chirurgie 48 (1921), 731.

Scopolamine, Scopine and Scopoline[1]

Up to the present time the primary cleavage-product, that is, the fundamental component of scopolamine has remained unknown (Gadamer and Hammer; Hess and Wahl). Willstätter[2] succeeded in obtaining this compound, which he calls scopine, by hydrolyzing scopolamine in a weak alkaline medium. Scopoline, which has long been known, is a secondary formation due to rearrangement of the α-oxide-ring of scopine forming the more stable γ-oxide-ring of scopoline, as shown in the formulae:



Scopine



Scopoline

Scopine may be obtained from scopolamine, by using pancreatic lipase as a mild hydrolytic agent in presence of ammonia-ammonium chloride buffer and activated with albumin and calcium chloride. Hydrolysis of the same character takes place also with ammonia-ammonium chloride alone. In one experiment a clear solution of 34 grams of free scopolamine stood for 9 days in a thermostat at 30° in 500 cc. of 0.5 n-ammonium chloride and 250 cc. of 0.5 n-ammonia solution. The solution was then concentrated *in vacuo* to 200 cc., 20 per cent. ammonia added, and 10 extractions made with an equal volume of chloroform. In this way 14 grams of unchanged scopolamine were recovered and 3.6 grams of nearly pure scopine were obtained from the fourth to the tenth extraction. An additional 1.4 gram of pure scopine was isolated from an oily mixture of the second and third fraction. When solutions of scopine in ether and chloroform were evaporated *in vacuo*, the alkaloid at once solidified to a crystalline mass, whereas it crystallized only with great difficulty when mixed with scopoline.

Scopine, $C_8H_{13}O_2N$, dissolves very easily in water, alcohol, acetone and chloroform; easily in ether and benzene; and with difficulty in petroleum ether. Crystallization takes place best from low-boiling petroleum ether in long needles that are stable in air and melt sharply at 70°. This base is optically inactive. The stability of scopine is greater in presence of acids than of alkalies. No change occurred as a result of standing for 24 hours in n-hydrochloric acid at room temperature. On the other hand, 24 hours standing at the same temperature with n-potassium hydroxide sufficed to bring about almost complete isomerization of scopine, that is, its conversion into scopoline. Likewise scopoline passed over, when scopine was distilled under ordinary pressure at 248°, mixed to be sure with some scopine. The residue in the flask consisted of pure scopoline. Even at 200° considerable scopoline is formed from scopine.

[1] Supplement to the Article "Scopolamine" on page 182.

[2] R. Willstätter and E. Berner: Hydrolysis of Scopolamine. Ber. d. Deutsch. chem. Ges. 56 (1923), 1079.

Colorimetric Estimation of Minute Quantities of Hydrogen Sulphide in Water and Air[1]

Formation of methylene blue by the Caro-E.Fischer reaction is as delicate as it is specific and may be used to advantage in determining colorimetrically even fractions of a thousandth of a mg. of hydrogen sulphide in water and air. In using the Autenrieth-Koenigsberger wedge-colorimeter (page 468), an empty glass-stoppered wedge is filled with any very dilute aqueous methylene blue solution. The comparison-wedge is then adjusted by means of a freshly prepared, dilute hydrogen sulphide water, the exact content of the gas having been previously accurately determined by titration with 0.1 n-iodine solution with starch solution as indicator. To calibrate the comparison-wedge, a few mg. of p-amino-dimethyl-aniline hydrochloride are dissolved cold in a little water in a 200 cc. graduated flask. Then add 10–20 cc. of titrated hydrogen sulphide water, $\frac{1}{50}$ of the total volume of concentrated hydrochloric acid, and finally a few drops of ferric chloride solution. Shake the mixture and allow it to stand for 20–25 minutes in the closed flask. Formation of methylene blue is then complete and the maximum coloration is reached. The smell of hydrogen sulphide has gone. If not, add some more p-amino-dimethyl-aniline and ferric chloride. Then dilute the blue solution with water to the 200 cc. mark and shake. By means of a burette or capillary pipette, measure each time a different volume, that is, 1, 2, 3 cc., of this solution, always diluting to the same volume, for example, 10 cc. Then use this solution in the colorimeter and determine on the scale the point at which this color solution and that in the comparison-wedge have the same strength. Calibration of a comparison-wedge gave the following values:

Color solution in cc.:	10	9	8	7	6	5	4	3	2	1
Water in cc	0	1	2	3	4	5	6	7	8	9
Hydrogen sulphide (thousandths of a mg. per cc.)	2.3	2.07	1.84	1.61	1.38	1.15	0.92	0.69	0.46	0.23
Scale reading for equal color strength	7	16	25	35	44	53	62	71	80	90

To construct a calibration curve, record quantities of hydrogen sulphide in 1 cc., expressed in thousandths of a mg., upon the abscissae and the corresponding scale readings for equal color strength upon the ordinates. The curve connecting these points is the calibration curve of the comparison-wedge. The following results show the accuracy of this method:

Taken	0.08	0.115	0.023	0.231	0.693	mg. of H_2S
Found	0.071	0.149	0.023	0.210	0.671	mg. of H_2S

Notes.—Unless the solution in which methylene blue is formed is strongly acid with hydrochloric acid, a red color will appear. When the color has once reached a maximum, as it does after 20–30 minutes, the solution retains the blue color unchanged in a closed flask for a long time.

[1] According to the unpublished work of W. Autenrieth and F. Breh.

The quantity of hydrogen sulphide in an aqueous solution may be determined in the same manner as described above for the standardization of the comparison-wedge.

Estimation of Hydrogen Sulphide in Air

Hydrogen sulphide may be absorbed from air by means of two or three wash-bottles that are connected and about one-third full of dilute sodium hydroxide solution. The air to be examined is drawn through this solution by suction. The quantity of air used is most conveniently measured by a gas-meter which must first be calibrated. To do this, force the air from a gasometer through the gas-meter and collect in a graduated measuring cylinder. In this way the height of water in the gas-meter at which, for example, 1 liter of air passes through in 5 minutes is ascertained. Then place a scale on the gas-meter, the divisions of which fixed experimentally show in every instance the state of the meter for the quantity of air passing through in the unit of time.

Experiment.—At mark II, 1 liter of air passes through the calibrated gas-meter in 6 minutes. Consequently 20 liters will pass through in 2 hours. Stop the experiment at the end of that time and determine hydrogen sulphide in the absorbing solution by the method used in calibrating the comparison-wedge. Special care must be taken to make sure that the solution used in forming methylene blue is strongly acid with hydrochloric acid. From this experiment it was determined that 20 liters of air contained 0.063 mg. of hydrogen sulphide. The sodium hydroxide solution in the third wash-bottle was examined by itself and found to give no methylene blue. Hydrogen sulphide therefore in 20 liters of air was completely absorbed in the first two wash-bottles. If the current air is too rapid, traces of hydrogen sulphide, such as may occur in the air of the laboratory, may pass through the wash-bottles without being absorbed.

Notes.—The meter may be read more conveniently, if the water used to fill it is colored by some indifferent dyestuff. To regulate the current of air, so that the height of water in the meter remains constant, a screw-clamp should be placed in front of the meter and one behind it, the latter being in front of the pump. By adjusting these screw-clamps properly, the current of air may be made to flow at the desired rate.

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